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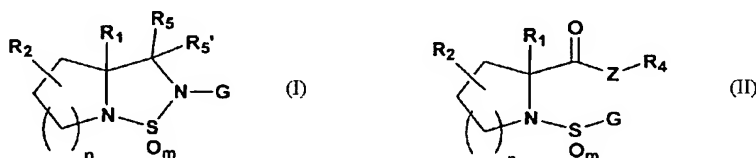
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(54) Title: SULFONYLPYRROLODINE MODULATORS OF ANDROGEN RECEPTOR FUCTION AND METHOD



(57) Abstract: Compounds are provided which are useful in the treatment of androgen receptor-associated conditions, such as age-related diseases, which compounds have the structure of formulae (I) or (II); wherein  $R_1$  is hydrogen (H), alkyl or substituted alkyl, alkenyl or substituted alkenyl, arylalkyl or substituted arylalkyl,  $CO_2R_{4a}$ ,  $CONR_{4a}R_{4b}$ , and  $CH_2OR_{4a}$ ;  $R_2$  is hydrogen (H),  $OR_3$ ,  $SR_3$ , halo,  $NHR_3$ ,  $NHCOR_{4c1}$ ,  $NHCO_2R_{4c1}$ ,  $NHCONR_{4c}R_{4d}$  and  $NHSO_2R_{4c}$ ;  $R_3$  in each functional group is hydrogen (H), alkyl or substituted alkyl,  $CHF_2$ ,  $CF_3$  and  $COR_{4c}$ ;  $R_4$ ,  $R_{4a}$ ,  $R_{4b}$ ,  $R_{4c}$ ,  $R_{4c1}$ ,  $R_{4d}$ ,  $R_{4e}$ ,  $R_{4f}$ ,  $R_{4g}$ , or  $R_{4h}$  in each functional group are the same or different and are hydrogen(H), alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, arylalkyl or substituted arylalkyl, aryl or substituted aryl, or heteroaryl or substituted heteroaryl;  $R_5$  and  $R_5'$  are the same or different and are hydrogen(H), alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, arylalkyl or substituted arylalkyl, aryl or substituted aryl, or heteroaryl or substituted heteroaryl, wherein at least one of  $R_5$  and  $R_5'$  is hydrogen, or  $R_5$  and  $R_5'$  taken together form a double bond with oxygen (O), sulfur (S),  $NR_7$  or  $CR_7R_7$ ; where  $R_7$  and  $R_7$  are as defined herein; G is an aryl, heterocyclo or heteroaryl group, wherein said group is mono- or polycyclic, and which is optionally substituted; and n is an integer of 1 or 2, m is an integer of 1 or 2, Z is oxygen (-O-) or  $NR_{4h}$ , a prodrug ester, all stereoisomers thereof and a pharmaceutically acceptable salt. A method for treating androgen receptor-associated conditions such as age-related diseases is also provided.



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## **SULFONYLPYRROLIDINE MODULATORS OF ANDROGEN RECEPTOR FUNCTION AND METHOD**

### **RELATED APPLICATIONS**

- 5           This application claims priority benefit under Title 35 § 119(e) of United States Provisional Application No. 60/541,869, filed February 4, 2004, the contents of which are herein incorporated by reference.

### **FIELD OF THE INVENTION**

- 10           The present invention relates to bicyclic compounds, methods of using such compounds in the treatment of androgen receptor-associated conditions, such as age-related diseases, for example sarcopenia, and to pharmaceutical compositions containing such compounds.

### **BACKGROUND OF THE INVENTION**

- 15           Nuclear hormone receptors (NHR's) constitute a large super-family of structurally-related and sequence-specific gene regulators scientists have named "ligand-dependent transcription factors." R.M. Evans, *Science*, 240:889 (1988). The steroid binding NHR's (SB-NHR's) form a recognized subset of the NHR's, including  
20           the progesterone receptor (PR), androgen receptor (AR), estrogen receptor (ER), glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). The conventional nuclear hormone receptors are generally transactivators in the presence of ligand, which selectively bind to the NHR in a way that effects gene transcription. In the absence of a corresponding ligand, some of the orphan receptors behave as if they are  
25           transcriptionally inert. Others, however, behave as either constitutive activators or repressors. These orphan nuclear hormone receptors are either under the control of ubiquitous ligands that have not been identified, or do not need to bind ligand to exert these activities.

- 30           The AR is a ligand-activated transcriptional regulatory protein that mediates induction of male sexual development and function through its activity with endogenous androgens. In addition, androgens are associated with male and female maintenance of muscle mass and strength, bone mass and erythropoiesis. Androgens,

such as testosterone, also play an important role in many physiological processes, such as differentiation of male internal and external genitalia, development and maintenance of male secondary sexual characteristics (e.g., the development of prostate, seminal vesicles, penis, scrotum, skeletal muscle, redistribution of body fat, stimulation of long bone growth, closure of epiphyses, development of male hair growth pattern and enlargement of larynx), the maintenance of sexual behavior and function (e.g., libido and potency) and spermatogenesis (in man).

As one ages, the serum androgen concentration in the body declines. The age dependent decline in androgens is associated with changes in body composition for men and women, such as a lower percentage of muscle mass and an increase in body fat, e.g., sarcopenia. In this regard, modulation of the AR gene can have an impact on the physiological effects associated with androgen production. However, the effectiveness of known modulators of steroid receptors is often tempered by their undesired side-effect profile, particularly during long-term administration. For example, the administration of synthetic androgens has been associated with liver damage, prostate cancer, adverse effects on male sexual function and adverse effects associated with cardiovascular and erythropoietic function.

Numerous synthetically-derived steroidal and non-steroidal agonists and antagonists have been described for the members of the SB-NHR family. Many of these agonist and antagonist ligands are used clinically in man to treat a variety of medical conditions. RU486 (mifepristone) is an example of a synthetic antagonist of the PR, which is utilized as a birth control agent (Vegeto et al., *Cell* 69: 703-713 (1992)). Flutamide is an example of an antagonist of the AR, which is utilized for the treatment of prostate cancer (Neri et al, *Endo.* 91, 427-437 (1972)). Tamoxifen is an example of a tissue-selective modulator of the ER function, that is used in the treatment of breast cancer (Smigel *J. Natl. Cancer Inst.* 90, 647-648 (1998)). Tamoxifen can function as an antagonist of the ER in breast tissue while acting as an agonist of the ER in bone (Grese et al., *Proc. Natl. Acad. Sci. USA* 94, 14105-14110 (1997)). Because of the tissue-selective effects seen for Tamoxifen, this agent, and agents like it, are referred to as tissue-selective estrogen receptor modulators. In addition to synthetically-derived non-endogenous ligands, non-endogenous ligands for NHR's can be obtained from food sources (Regal et al., *Proc. Soc. Exp. Biol. Med.*



223, 372-378 (2000) and Hempstock et al., *J. Med. Food* 2, 267-269 (1999)). The flavanoid phytoestrogens are an example of an unnatural ligand for SB-NHR's that are readily obtained from a food source such as soy (Quella et al., *J. Clin. Oncol.* 18, 1068-1074 (2000) and Banz et al., *J. Med. Food* 2, 271-273 (1999)). The ability to  
5 modulate the transcriptional activity of an individual NHR by the addition of a small molecule ligand, makes these receptors ideal targets for the development of pharmaceutical agents for a variety of disease states.

As mentioned above, non-natural ligands can be synthetically engineered to serve as modulators of the function of NHR's. In the case of SB-NHR's, engineering  
10 of an unnatural ligand can include the identification of a core structure which mimics the natural steroid core system. This can be achieved by random screening against several SB-NHR's, or through directed approaches using the available crystal structures of a variety of NHR ligand binding domains (Bourguet et al., *Nature* 375, 377-382 (1995), Brzozowski, et al., *Nature* 389, 753-758 (1997), Shiau et al., *Cell* 95,  
15 927-937 (1998) and Tanenbaum et al., *Proc. Natl. Acad. Sci. USA* 95, 5998-6003 (1998)). Differential substitution about such a steroid mimic core can provide agents with selectivity for one receptor versus another. In addition, such modifications can be employed to obtain agents with agonist or antagonist activity for a particular SB-NHR. Differential substitution about the steroid mimic core can result in the  
20 formation of a series of high affinity agonists and antagonists with specificity for, for example, ER versus PR versus AR versus GR versus MR. Such an approach of differential substitution has been reported, for example, for quinoline based modulators of steroid NHRs in Hamann et. al., *J. Med. Chem.*, 41, 623 (1998); Hamann et. al., *J. Med. Chem.* 42, 210 (1999); WO 9749709; US 5696133; US  
25 5696130; US 5696127; US 5693647; US 5693646; US 5688810; US 5688808 and WO 9619458, all incorporated herein by reference.

Accordingly, identification of compounds which have good specificity for one or more steroid receptors, but which have reduced or no cross-reactivity for other steroid or intracellular receptors, would be of significant value in the treatment of  
30 male and female hormone-responsive diseases. There is, therefore, a need in the art for the identification of selective modulators of the steroid binding nuclear hormone receptors, particularly non-steroidal, non-toxic tissue selective androgen receptor

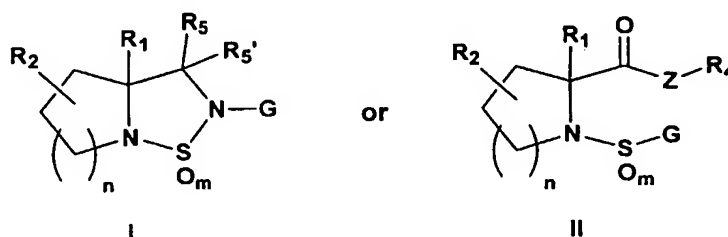
modulators, which activate the androgen receptor in skeletal muscle while demonstrating limited or neutral effect on other androgen responsive (e.g., prostate) tissues.

## 5

## SUMMARY OF THE INVENTION

In accordance with illustrative embodiments and demonstrating features of the present invention, compounds are provided which are capable of modulating the function of a nuclear hormone receptor. Preferably the compounds are selective androgen receptor modulators, and have the general formula I or II

10



wherein

15  $R_1$  is hydrogen (H), alkyl or substituted alkyl, alkenyl or substituted alkenyl, arylalkyl or substituted arylalkyl,  $CO_2R_{4a}$ ,  $CONR_{4a}R_{4b}$ , or  $CH_2OR_{4a}$ ;

$R_2$  is hydrogen (H),  $OR_3$ ,  $SR_3$ , halo,  $NHR_3$ ,  $NHCOR_{4c1}$ ,  $NHCO_2R_{4c1}$ ,  $NHCONR_{4c}R_{4d}$  or  $NHSO_2R_{4c}$ ;

R<sub>3</sub> in each functional group is independently hydrogen (H), alkyl, substituted alkyl, CHF<sub>2</sub>, CF<sub>3</sub> or COR<sub>4e</sub>;

20  $R_4, R_{4a}, R_{4b}, R_{4c}, R_{4c_1}, R_{4d}, R_{4e}, R_{4f}, R_{4g},$  and  $R_{4h}$  in each functional group are the same or different and are each independently hydrogen(H), alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, arylalkyl or substituted arylalkyl, aryl or substituted aryl, or heteroaryl or substituted heteroaryl;

25             $R_5$  and  $R_5'$  are the same or different and are each independently hydrogen(H), alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, arylalkyl or substituted arylalkyl, aryl or substituted aryl, or heteroaryl or substituted heteroaryl, wherein at least one of  $R_5$  and

R<sub>5</sub>' is hydrogen, or R<sub>5</sub> and R<sub>5</sub>' taken together can form a double bond with oxygen (O), sulfur (S), NR<sub>7</sub> or CR<sub>7</sub>R<sub>7</sub>';

R<sub>7</sub> and R<sub>7</sub>' in each functional group are the same or different and are each independently hydrogen(H), OR<sub>4</sub>, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, arylalkyl or substituted arylalkyl, aryl or substituted aryl, or heteroaryl or substituted heteroaryl;

G is an aryl, heterocyclo or heteroaryl group, wherein said group is mono- or polycyclic, and which is optionally substituted with one or more substituents selected from the group consisting of hydrogen, halo, CN, CF<sub>3</sub>, OR<sub>4f</sub>, CO<sub>2</sub>R<sub>4f</sub>, NR<sub>4f</sub>R<sub>4g</sub>, CONR<sub>4f</sub>R<sub>4g</sub>, CH<sub>2</sub>OR<sub>4f</sub>, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, arylalkyl or substituted arylalkyl, aryl or substituted aryl, or heteroaryl or substituted heteroaryl; and

n is an integer of 1 or 2;  
m is an integer of 1 or 2;  
Z is oxygen (-O-) or NR<sub>4h</sub>.

The definition of formula I above includes of all prodrug esters, stereoisomers and pharmaceutically acceptable salts of formula I.

The compounds of formula I modulate the function of the nuclear hormone receptors, particularly the androgen receptor, and include compounds which are, for example, selective agonists, partial agonists, antagonists or partial antagonists of the androgen receptor. Preferably the compounds of formula I possess activity as agonists of the androgen receptor and may be used in the treatment of diseases or disorders associated with androgen receptor activity, such as maintenance of muscle strength and function (e.g., in the elderly); reversal or prevention of frailty or age-related functional decline ("ARFD") in the elderly (e.g., sarcopenia); prevention of catabolic side effects of glucocorticoids; prevention and treatment of reduced bone density or growth (e.g., osteoporosis and osteopenia); treatment of chronic fatigue syndrome (CFS); chronic myalgia; treatment of acute fatigue syndrome and muscle loss following elective surgery (e.g., post-surgical rehabilitation); treatment of urinary

incontinence, acceleration of wound healing; accelerating bone fracture repair (such as accelerating the recovery of hip fracture patients); treatment of wasting secondary to fractures and wasting in connection with chronic obstructive pulmonary disease (COPD), chronic liver disease, AIDS, weightlessness, cancer cachexia, burn and  
5 trauma recovery, chronic catabolic state (e.g., coma), eating disorders (e.g., anorexia) and chemotherapy.

The present invention provides for compounds of formula I, pharmaceutical compositions employing such compounds and for methods of using such compounds. In particular, the present invention provides a pharmaceutical composition comprising  
10 a therapeutically effective amount of a compound of formula I, alone or in combination with a pharmaceutically acceptable carrier.

Further, in accordance with the present invention, a method is provided for preventing, inhibiting or treating the progression or onset of diseases or disorders associated with nuclear hormone receptors, particularly, the androgen receptor, such  
15 as the diseases or disorders defined above and hereinafter, wherein a therapeutically effective amount of a compound of formula I is administered to a mammalian, i.e., human, patient in need of treatment.

The compounds of the invention can be used alone, in combination with other compounds of the present invention, or in combination with one or more other  
20 agent(s) active in the therapeutic areas described herein.

In addition, a method is provided for preventing, inhibiting or treating the diseases as defined above and hereinafter, wherein a therapeutically effective amount of a combination of a compound of formula I and another type of therapeutic agent, is administered to a human patient in need of treatment.

25

Preferred are compounds of formula I wherein

R<sub>1</sub> is hydrogen (H) or alkyl;

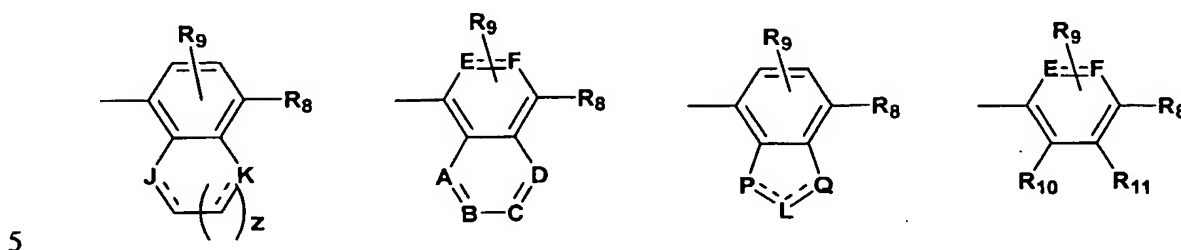
R<sub>2</sub> is hydroxyl (OH);

R<sub>5</sub> and R<sub>5</sub>' are hydrogen or are taken together form a double bond with oxygen  
30 (O) or sulfur (S); and

n is 1,

m is 2.

Further preferred embodiments include compounds of formula I where G is selected from:



wherein

$R_8$ ,  $R_9$ ,  $R_{10}$  and  $R_{11}$  in each functional group are each independently selected from the group consisting of hydrogen (H),  $\text{NO}_2$ , CN,  $\text{CF}_3$ ,  $\text{OR}_{4i}$ ,  $\text{CO}_2\text{R}_{4i}$ ,  $\text{NR}_{4i}\text{R}_{4j}$ ,  
 10  $\text{CONR}_{4i}\text{R}_{4j}$ ,  $\text{CH}_2\text{OR}_{4i}$ , alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, arylalkyl or substituted arylalkyl, aryl or substituted aryl, or heteroaryl or substituted heteroaryl;

A to F is each independently selected from N or  $\text{CR}_{1a}$ ;

J, K, L, P and Q are each independently selected from  $\text{NR}_{12}$ , O, S, SO,  $\text{SO}_2$  or  
 15  $\text{CR}_{12}\text{R}_{12}'$ ;

$R_{12}$  and  $R_{12}'$  in each functional group are each independently selected from a bond or  $\text{R}_{1b}$ ;

$R_{1a}$  and  $R_{1b}$  are each independently selected from H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, arylalkyl or substituted arylalkyl,  $\text{CO}_2\text{R}_{4k}$ ,  $\text{CONR}_{4k}\text{R}_{4l}$   
 20 and  $\text{CH}_2\text{OR}_{4k}$ ; and

z is an integer of 0 or 1,

$R_{4i}$ ,  $R_{4j}$ ,  $R_{4k}$  and  $R_{4l}$  are the same or different and are independently hydrogen(H), alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, arylalkyl or substituted  
 25 arylalkyl, aryl or substituted aryl, or heteroaryl or substituted heteroaryl;

Preferred are compounds where  $R_8$  is CN.

**DETAILED DESCRIPTION OF THE INVENTION**

The following abbreviations are employed herein:

- Chiralpak<sup>®</sup> = Trademark of Chiral Technologies, Inc. Eaton, PA  
DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene
- 5    AcOH = acetic acid  
     DMPU = 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone  
     EtOAc = ethyl acetate  
     HPLC = high performance liquid chromatography  
     MeOH = methanol
- 10   MS or Mass Spec = mass spectrometry  
     YMC<sup>®</sup> = trademark of YMC Co, Ltd., Kyoto, Japan  
     CuBr = copper(I) bromide  
     CuCN = copper(I) cyanide  
     CsF = cesium fluoride
- 15   Et<sub>3</sub>N = triethylamine  
     DCC = 1,3-dicyclohexylcarbodiimide  
     DEAD = diethyl azodicarboxylate  
     LDA = lithium diisopropylamide  
     NMP = 1-methyl-2-pyrrolidinone
- 20   KOH = potassium hydroxide  
     Pd/C = palladium on activated charcoal  
     TFA = trifluoroacetic acid  
     THF = tetrahydrofuran  
     mp. = melting point
- 25   min = minute(s)  
     h = hour(s)  
     L = liter  
     mL = milliliter  
      $\mu$ L = microliter
- 30   g = gram(s)  
     mg = milligram(s)  
     mol = moles

mmol = millimole(s)

nM = nanomolar

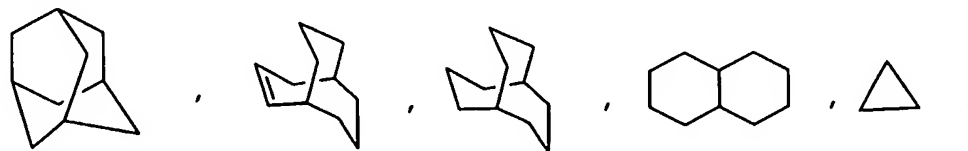
rt = room temperature

- 5           The following definitions apply to the terms as used throughout this specification, unless otherwise limited in specific instances.

As used herein, the term "alkyl" denotes branched or unbranched hydrocarbon chains, preferably having about 1 to about 8 carbons, such as, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl, tert-butyl, 2-methylpentyl, pentyl, hexyl, 10    iso-hexyl, heptyl, 4,4-dimethyl pentyl, octyl, 2,2,4-trimethylpentyl and the like. "Substituted alkyl" includes an alkyl group optionally substituted with one or more functional groups which are attached commonly to such chains, such as, hydroxyl, bromo, fluoro, chloro, iodo, mercapto or thio, cyano, alkylthio, heterocyclyl, aryl, heteroaryl, carboxyl, carbalkoyl, alkyl, alkenyl, nitro, amino, alkoxyl, amido, and the 15    like to form alkyl groups such as trifluoro methyl, 3-hydroxyhexyl, 2-carboxypropyl, 2-fluoroethyl, carboxymethyl, cyanobutyl and the like.

Unless otherwise indicated, the term "cycloalkyl" as employed herein alone or as part of another group includes saturated or partially unsaturated (containing 1 or more double bonds) cyclic hydrocarbon groups containing 1 to 3 rings, including 20    monocyclicalkyl, bicyclicalkyl and tricyclicalkyl, containing a total of 3 to 20 carbons forming the rings, preferably 3 to 10 carbons, forming the ring and which may be fused to 1 or 2 aromatic rings as described for aryl, which include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclodecyl and cyclododecyl, cyclohexenyl,

25



"Substituted cycloalkyl" includes a cycloalkyl group optionally substituted with 1 or more substituents such as halogen, alkyl, alkoxy, hydroxy, aryl, aryloxy,

arylalkyl, cycloalkyl, alkylamido, alkanoylamino, oxo, acyl, arylcarbonylamino, amino, nitro, cyano, thiol and/or alkylthio and/or any of the substituents included in the definition of "substituted alkyl."

Unless otherwise indicated, the term "alkenyl" as used herein by itself or as  
5 part of another group refers to straight or branched chain radicals of 2 to 20 carbons, preferably 2 to 12 carbons, and more preferably 2 to 8 carbons in the normal chain, which include one or more double bonds in the normal chain, such as vinyl, 2-propenyl, 3-butenyl, 2-butenyl, 4-pentenyl, 3-pentenyl, 2-hexenyl, 3-hexenyl, 2-heptenyl, 3-heptenyl, 4-heptenyl, 3-octenyl, 3-nonenyl, 4-decenyl, 3-undecenyl, 4-  
10 dodecenyl, 4,8,12-tetradecatrienyl, and the like. "Substituted alkenyl" includes an alkenyl group optionally substituted with one or more substituents, such as the substituents included above in the definition of "substituted alkyl" and "substituted cycloalkyl."

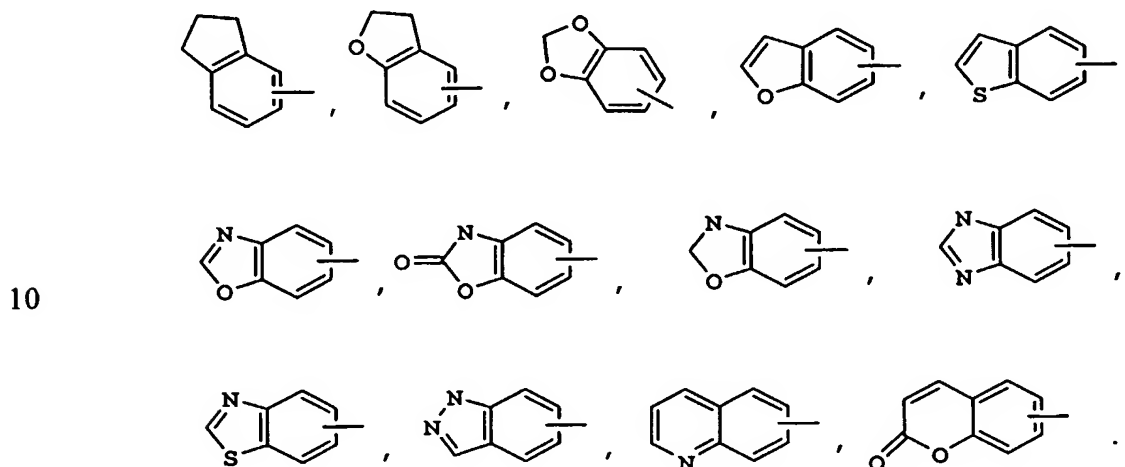
Unless otherwise indicated, the term "alkynyl" as used herein by itself or as  
15 part of another group refers to straight or branched chain radicals of 2 to 20 carbons, preferably 2 to 12 carbons and more preferably 2 to 8 carbons in the normal chain, which include one or more triple bonds in the normal chain, such as 2-propynyl, 3-butynyl, 2-butynyl, 4-pentynyl, 3-pentynyl, 2-hexynyl, 3-hexynyl, 2-heptynyl, 3-heptynyl, 4-heptynyl, 3-octynyl, 3-nonyl, 4-decynyl, 3-undecynyl, 4-dodecynyl and  
20 the like. "Substituted alkynyl" includes an alkynyl group optionally substituted with one or more substituents, such as the substituents included above in the definition of "substituted alkyl" and "substituted cycloalkyl."

The terms "arylalkyl", "arylalkenyl" and "arylalkynyl" as used alone or as part of another group refer to alkyl, alkenyl and alkynyl groups as described above having  
25 an aryl substituent. Representative examples of arylalkyl include, but are not limited to, benzyl, 2-phenylethyl, 3-phenylpropyl, phenethyl, benzhydryl and naphthylmethyl and the like. "Substituted arylalkyl" includes arylalkyl groups wherein the aryl portion is optionally substituted with one or more substituents, such as the substituents included above in the definition of "substituted alkyl" and "substituted  
30 cycloalkyl."

The term "halogen" or "halo" as used herein alone or as part of another group refers to chlorine, bromine, fluorine, and iodine.

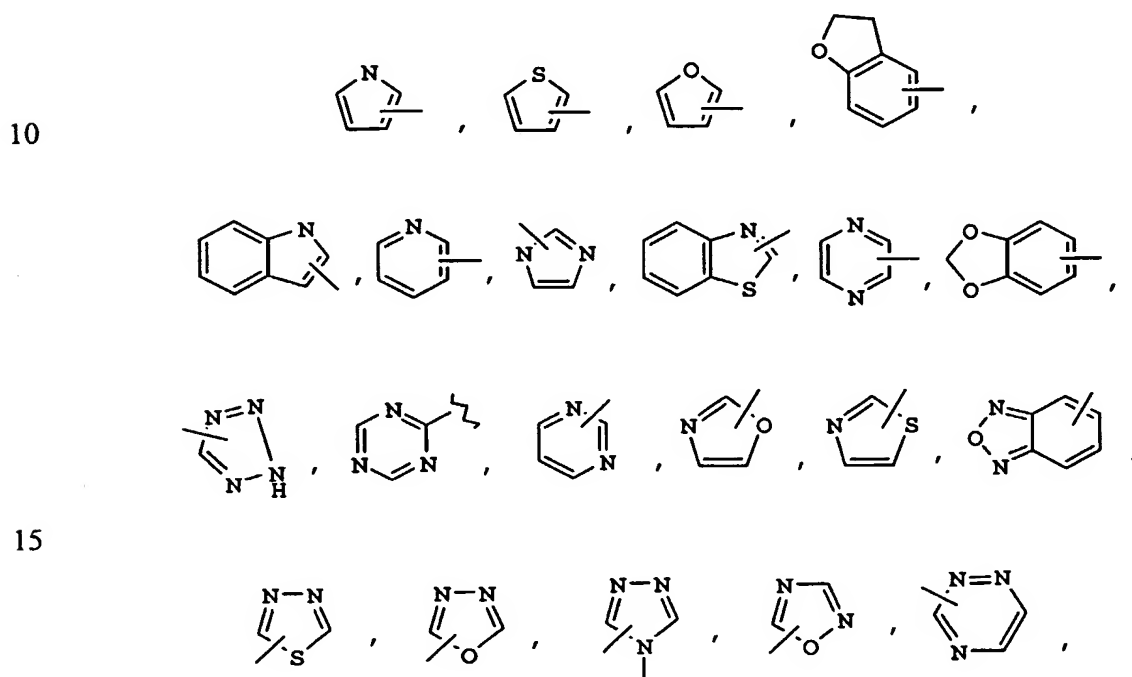


Unless otherwise indicated, the term "aryl" or "Ar" as employed herein alone or as part of another group refers to monocyclic and polycyclic aromatic groups containing 6 to 10 carbons in the ring portion (such as phenyl or naphthyl including 1-naphthyl and 2-naphthyl) and may optionally include one to three additional rings fused to a carbocyclic ring or a heterocyclic ring (such as aryl, cycloalkyl, heteroaryl or cycloheteroalkyl rings), for example



"Substituted aryl" includes an aryl group optionally substituted with one or more functional groups, such as halo, haloalkyl, alkyl, haloalkyl, alkoxy, haloalkoxy, alkenyl, trifluoromethyl, trifluoromethoxy, alkynyl, cycloalkyl-alkyl, cycloheteroalkyl, cycloheteroalkylalkyl, aryl, heteroaryl, arylalkyl, aryloxy, aryloxyalkyl, arylalkoxy, alkoxy carbonyl, aryl carbonyl, arylalkenyl, aminocarbonylaryl, arylthio, arylsulfinyl, arylazo, heteroarylalkyl, heteroarylalkenyl, heteroarylheteroaryl, heteroaryloxy, hydroxy, nitro, cyano, amino, substituted amino wherein the amino includes 1 or 2 substituents (which are alkyl, aryl or any of the other aryl compounds mentioned in the definitions), thiol, alkylthio, arylthio, heteroarylthio, arylthioalkyl, alkoxyarylthio, alkylcarbonyl, arylcarbonyl, alkylaminocarbonyl, arylaminocarbonyl, alkoxy carbonyl, aminocarbonyl, alkylcarbonyloxy, arylcarbonyloxy, alkylcarbonylamino, arylcarbonylamino, arylsulfinyl, arylsulfinylalkyl, arylsulfonylamino or arylsulfonaminocarbonyl and/or any of the alkyl substituents set out herein.

Unless otherwise indicated, the term "heteroaryl" as used herein alone or as part of another group refers to a 5- or 7- membered aromatic ring which includes 1, 2, 3 or 4 hetero atoms such as nitrogen, oxygen or sulfur and such rings fused to an aryl, cycloalkyl, heteroaryl or heterocycloalkyl ring (e.g. benzothiophenyl, indolyl), and includes possible N-oxides. "Substituted heteroaryl" includes a heteroaryl group optionally substituted with 1 to 4 substituents, such as the substituents included above in the definition of "substituted alkyl" and "substituted cycloalkyl." Examples of heteroaryl groups include the following:



and the like.

The term "heterocyclo", heterocycle or heterocyclic ring, as used herein, represents an unsubstituted or substituted stable 5- to 7-membered monocyclic ring system which may be saturated or unsaturated, and which consists of carbon atoms and from one to four heteroatoms selected from N, O or S, and wherein the nitrogen and sulfur heteroatoms may optionally be oxidized, and the nitrogen heteroatom may optionally be quaternized. The heterocyclic ring may be attached at any heteroatom or carbon atom which results in the creation of a stable structure. Examples of such heterocyclic groups include, but is not limited to, piperidinyl, piperazinyl,

oxopiperazinyl, oxopiperidinyl, oxopyrrolidinyl, oxoazepinyl, azepinyl, pyrrolyl, pyrrolidinyl, furanyl, thienyl, pyrazolyl, pyrazolidinyl, imidazolyl, imidazoliny, imidazolidinyl, pyridyl, pyrazinyl, pyrimidinyl, pyridazinyl, oxazolyl, oxazolidinyl, isooxazolyl, isoxazolidinyl, morpholinyl, thiazolyl, thiazolidinyl, isothiazolyl, thiadiazolyl, tetrahydropyranyl, thiamorpholinyl, thiamorpholinyl sulfoxide, thiamorpholinyl sulfone, and oxadiazolyl.

The compounds of formula I can be present as salts, which are also within the scope of this invention. Pharmaceutically acceptable (i.e., non-toxic, physiologically acceptable) salts are preferred. If the compounds of formula I have, for example, at least one basic center, they can form acid addition salts. These are formed, for example, with strong inorganic acids, such as mineral acids, for example sulfuric acid, phosphoric acid or a hydrohalic acid, with strong organic carboxylic acids, such as alkanecarboxylic acids of 1 to 4 carbon atoms which are unsubstituted or substituted, for example, by halogen, for example acetic acid, such as saturated or unsaturated dicarboxylic acids, for example oxalic, malonic, succinic, maleic, fumaric, phthalic or terephthalic acid, such as hydroxycarboxylic acids, for example ascorbic, glycolic, lactic, malic, tartaric or citric acid, such as amino acids, (for example aspartic or glutamic acid or lysine or arginine), or benzoic acid, or with organic sulfonic acids, such as (C<sub>1</sub>-C<sub>4</sub>) alkyl or arylsulfonic acids which are unsubstituted or substituted, for example by halogen, for example methyl- or *p*-toluene- sulfonic acid. Corresponding acid addition salts can also be formed having, if desired, an additionally present basic center. The compounds of formula I having at least one acid group (for example COOH) can also form salts with bases. Suitable salts with bases are, for example, metal salts, such as alkali metal or alkaline earth metal salts, for example sodium, potassium or magnesium salts, or salts with ammonia or an organic amine, such as morpholine, thiomorpholine, piperidine, pyrrolidine, a mono, di or tri-lower alkylamine, for example ethyl, *tert*-butyl, diethyl, diisopropyl, triethyl, tributyl or dimethyl-propylamine, or a mono, di or trihydroxy lower alkylamine, for example mono, di or triethanolamine. Corresponding internal salts may furthermore be formed. Salts which are unsuitable for pharmaceutical uses but which can be employed, for example, for the isolation or purification of free compounds of formula I or their pharmaceutically acceptable salts, are also included.

Preferred salts of the compounds of formula I which contain a basic group include monohydrochloride, hydrogensulfate, methanesulfonate, phosphate or nitrate.

Preferred salts of the compounds of formula I which contain an acid group include sodium, potassium and magnesium salts and pharmaceutically acceptable  
5 organic amines.

The term "modulator" refers to a chemical compound with capacity to either enhance (e.g., "agonist" activity) or inhibit (e.g., "antagonist" activity) a functional property of biological activity or process (e.g., enzyme activity or receptor binding); such enhancement or inhibition may be contingent on the occurrence of a specific  
10 event, such as activation of a signal transduction pathway, and/or may be manifest only in particular cell types.

The term "prodrug esters" as employed herein includes esters and carbonates formed by reacting one or more hydroxyls of compounds of formula I with alkyl, alkoxy, or aryl substituted acylating agents employing procedures known to those  
15 skilled in the art to generate acetates, pivalates, methylcarbonates, benzoates and the like.

Any compound that can be converted in vivo to provide the bioactive agent (i.e., the compound of formula I) is a prodrug within the scope and spirit of the invention.

20 Various forms of prodrugs are well known in the art. A comprehensive description of prodrugs and prodrug derivatives are described in:

- (a) *The Practice of Medicinal Chemistry*, Camille G. Wermuth et al., Ch 31, (Academic Press, 1996);
- (b) *Design of Prodrugs*, edited by H. Bundgaard, (Elsevier, 1985);
- 25 (c) *A Textbook of Drug Design and Development*, P. Krogsgaard-Larson and H. Bundgaard, eds., Ch. 5, pgs, 113-191 (Harwood Academic Publishers, 1991).

Said references are incorporated herein by reference.

An administration of a therapeutic agent of the invention includes  
30 administration of a therapeutically effective amount of the agent of the invention. The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent to treat or prevent a condition treatable by administration of a

composition of the invention. That amount is the amount sufficient to exhibit a detectable therapeutic or preventative or ameliorative effect. The effect may include, for example, treatment or prevention of the conditions listed herein. The precise effective amount for a subject will depend upon the subject's size and health, the  
5 nature and extent of the condition being treated, recommendations of the treating physician, and the therapeutics or combination of therapeutics selected for administration. Thus, it is not useful to specify an exact effective amount in advance.

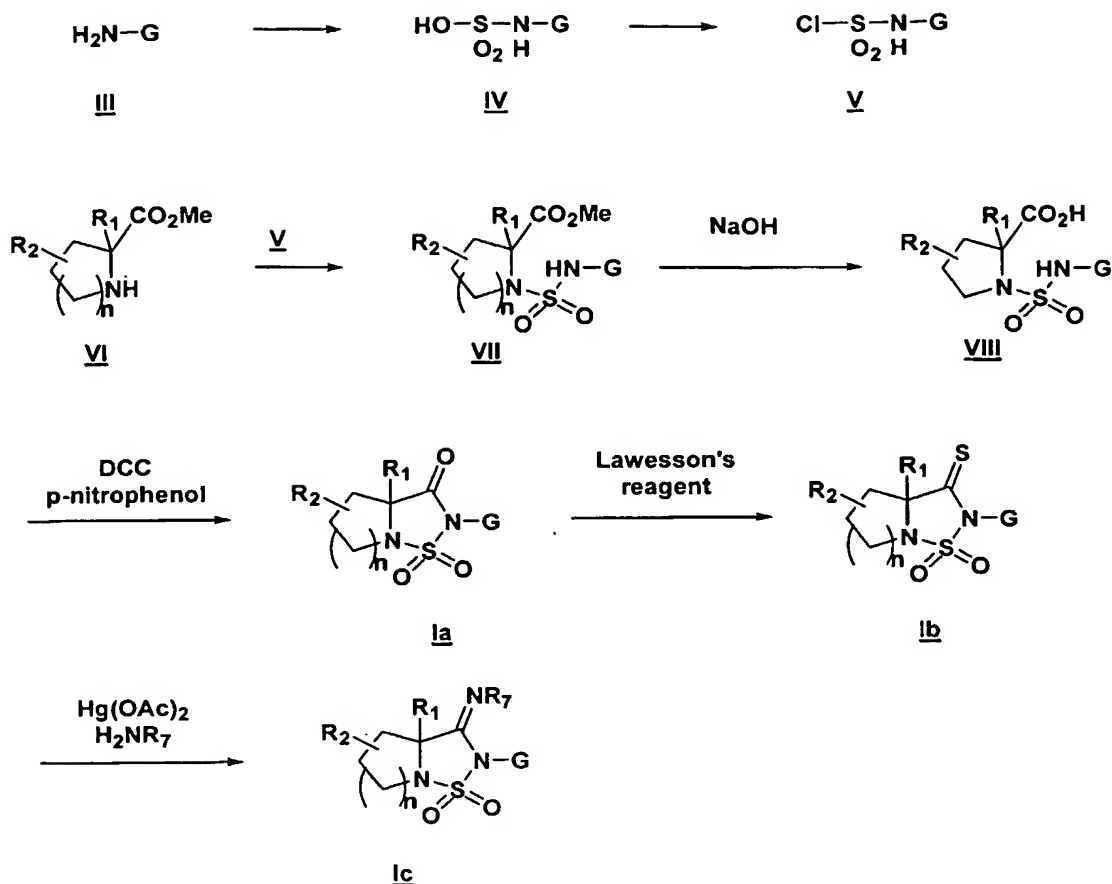
All stereoisomers of the compounds of the instant invention are contemplated, either in admixture or in pure or substantially pure form. The compounds of the  
10 present invention can have asymmetric centers at any of the carbon atoms including any one of the R substituents. Consequently, compounds of formula I can exist in enantiomeric or diastereomeric forms or in mixtures thereof. The processes for preparation can utilize racemates, enantiomers or diastereomers as starting materials. When diastereomeric or enantiomeric products are prepared, they can be separated by  
15 conventional methods for example, chromatographic, chiral HPLC or fractional crystallization.

The compounds of formula I of the present invention can be prepared as shown in the following reaction schemes and description thereof, as well as relevant published literature procedures that may be used by one skilled in the art. Exemplary  
20 reagents and procedures for these reactions appear hereinafter and in the working Examples.

Chemistry used to synthesize preferred compounds of the general structure I is shown below in **Schemes I–III**. Additional schemes will be added to enable the entire breadth of the genus.

25

## SCHEME I

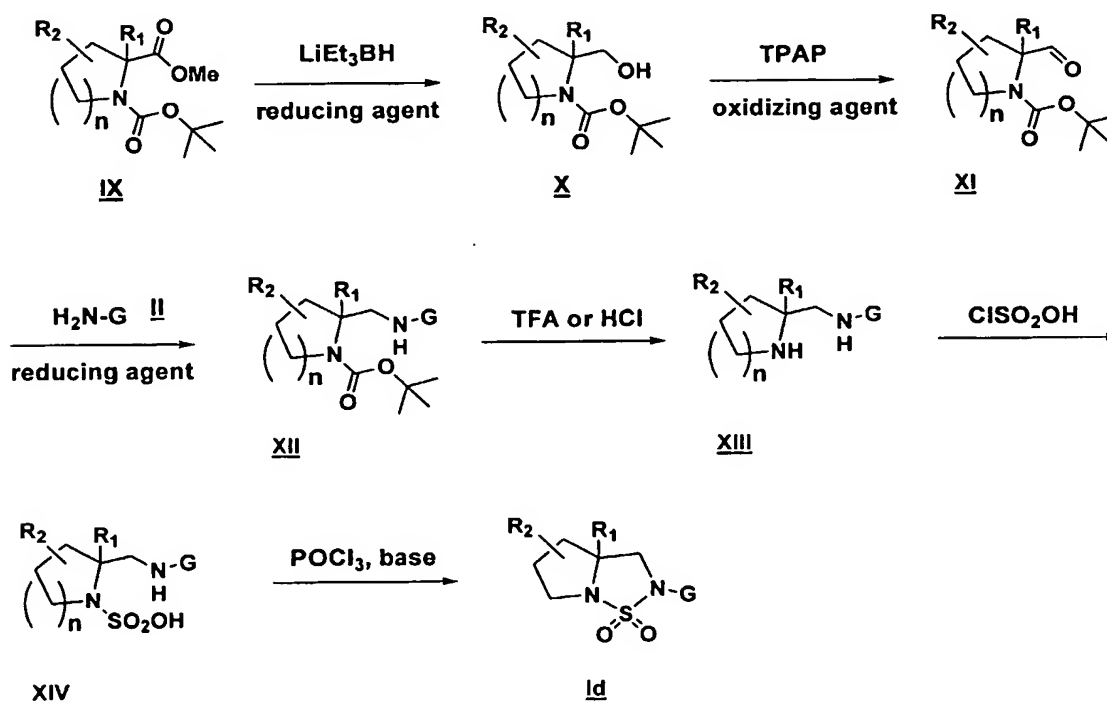


- 5 As illustrated in Scheme I, compounds of formula I can be prepared from intermediates of formula **III** and suitably protected intermediates of formula **VI**. Intermediates of formula **III** and **VI** can be obtained commercially, can be prepared by methods known in the literature or can be readily prepared by one skilled in the art. Treatment of intermediate **III** with chlorosulfonic acid provides intermediates of
- 10 formula **IV**. Intermediates **IV** can be treated with  $\text{PCl}_5$  or an equivalent reagent to provide intermediates of formula **V**. Combining intermediates of formula **V** and **VI** in the presence of a base, such as  $\text{Et}_3\text{N}$  or pyridine, gives intermediates of formula **VII**. Treatment of intermediates **VII** with  $\text{NaOH}$  or  $\text{NaOH}$  equivalent gives the carboxylic acid intermediates of formula **VIII**. The compound of formula **VIII** can be subjected
- 15 to acid coupling conditions, such as with  $\text{DCC}$  and  $p$ -nitrophenol, to yield compounds of formula **Ia** wherein  $\text{R}_5$  and  $\text{R}_5'$  are taken together to form a double bond to oxygen

(O). Compound **Ia** can be treated with a reagent such as Lawesson's Reagent to provide the compounds of formula **Ib** wherein  $R_5$  and  $R_{5'}$  are taken together to form a double bond to sulfur (S). Treatment of **Ib** with an amine in the presence of a divalent mercury salt such as  $Hg(OAc)_2$  gives compounds of formula **Ic** wherein  $R_5$  and  $R_{5'}$  are taken together to form a double bond to nitrogen (N). Where  $R_2$  is a suitably protected alcohol, thiol or amine, compounds of formula **Ia-c** can be treated by methods known in the literature or readily known by one skilled in the art to yield compounds of formula **Ia-c** wherein  $R_2$  is an alcohol, thiol or amine. Where  $R_2$  is an alcohol, thiol or amine, compounds of formula **Ia-c** can be treated by methods known in the literature or readily known by one skilled in the art to yield compounds of formula **Ia-c** wherein  $R_2$  is a hydrogen (H),  $OR_3$ ,  $SR_3$ , halo,  $NHR_3$ ,  $NHCOR_4$ ,  $NHCO_2R_4$ ,  $NHCONR_4R_4'$  and  $NHSO_2R_4$ .

## SCHEME II

15

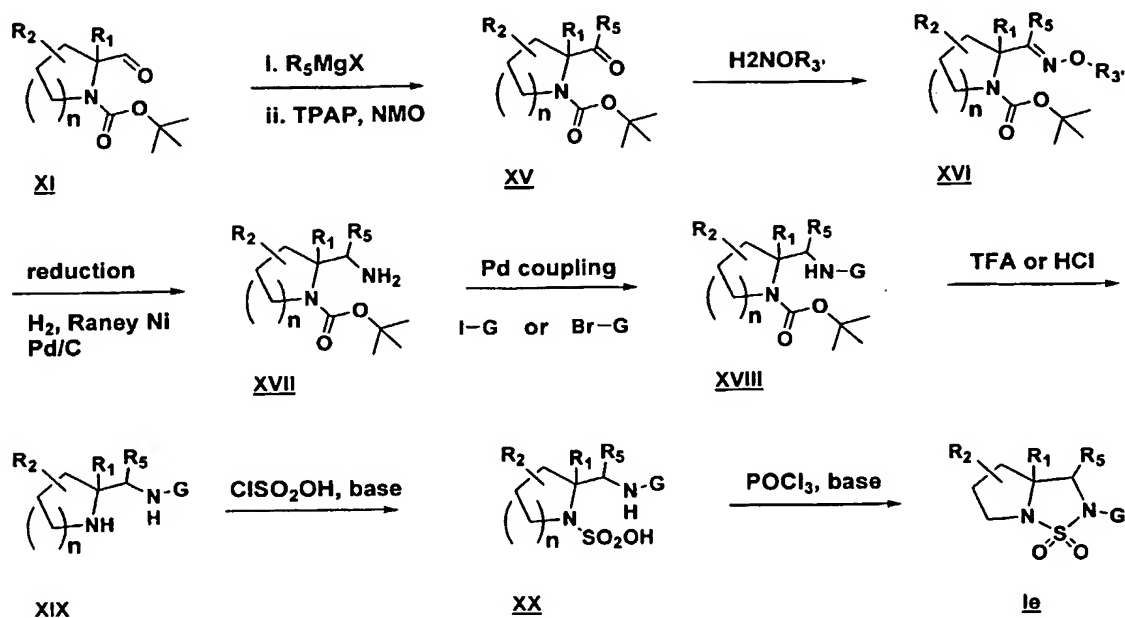


As illustrated in Scheme II, compounds of formula **I** can be prepared from suitably protected intermediates of formula **IX**. Intermediates of formula **IX** can be

obtained commercially, can be prepared by methods known in the literature or can be readily prepared by one skilled in the art. Treatment of **IX** with  $\text{LiEt}_3\text{BH}$  or another suitable reducing agent provides intermediates of formula of **X**. The intermediates of formula **X** can be treated with an oxidizing agent, such as TPAP with NMO, to yield  
5 intermediates of formula **XI**. Intermediates **XI** can be treated with an amine of formula  $\text{H}_2\text{NG}$  in the presence of a suitable reducing agent, such as  $\text{NaHB}(\text{OAc})_3$ , to yield intermediates of formula **XII**. Deprotection of intermediates **XII**, such as with TFA or HCl, gives intermediates of formula **XIII**. Intermediates **XIII** can be treated with chlorosulfonic acid or equivalent sulfonylating agent to yield intermediates **XIV**.  
10 Intermediates **XIV** can be treated with an activating agent, such as  $\text{POCl}_3$ , followed by a suitable base to provide compounds of formula **Id** wherein  $\text{R}_5$  and  $\text{R}_5'$  are both hydrogen (H). Where  $\text{R}_2$  is a suitably protected alcohol, thiol or amine, compounds of formula **Id** can be treated by methods known in the literature or readily known by one skilled in the art to yield compounds of formula **Id** wherein  $\text{R}_2$  is an alcohol, thiol or  
15 amine. Where  $\text{R}_2$  is an alcohol, thiol or amine, compounds of formula **Id** can be treated by methods known in the literature or readily known by one skilled in the art to yield compounds of formula **Id** wherein  $\text{R}_2$  is a hydrogen (H),  $\text{OR}_3$ ,  $\text{SR}_3$ , halo,  $\text{NHR}_3$ ,  $\text{NHCOR}_4$ ,  $\text{NHCO}_2\text{R}_4$ ,  $\text{NHCONR}_4\text{R}_4'$  and  $\text{NHSO}_2\text{R}_4$ .



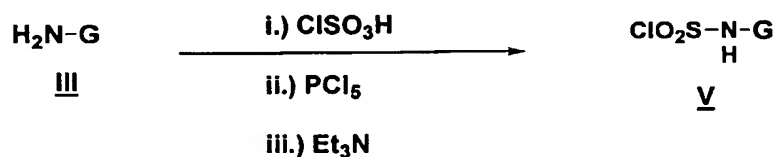
## SCHEME III



- 5 As illustrated in Scheme III, compounds of formula I can be prepared from intermediates of formula XI. Treatment of intermediates XI with an organometallic reagent by methods known in the literature or readily known by one skilled in the art followed by oxidation in with a suitable oxidizing agent, such as TPAP with NMO, provides intermediates of formula of XV. Treatment of intermediates of formula XV
- 10 with hydroxylamine or equivalent reagent gives intermediates of formula XVI. Intermediates XVI can be treated with a reducing agent, such as powdered Ni metal and/or Pd/C with hydrogen gas at elevated pressure, to provide intermediates of formula XVII. Coupling of intermediates XVII to an aryl- or heterocyclo- or heteroaryl halide such as I-G or Br-G, using Pd or Cu coupling methods or equivalent
- 15 methods, gives intermediates of formula XVIII. Deprotection of intermediates XVIII with TFA or HCl gives intermediates of formula XIX. Intermediates XIX can be treated with chlorosulfonic acid or other suitably activated sulfonic acid to yield intermediates XX. Intermediates XX can be treated with an activating agent, such as POCl<sub>3</sub>, followed by base to provide compounds of formula Ie. Where R<sub>2</sub> is a suitably
- 20 protected alcohol, thiol or amine, compounds of formula Ie can be treated by methods known in the literature or readily known by one skilled in the art to yield compounds

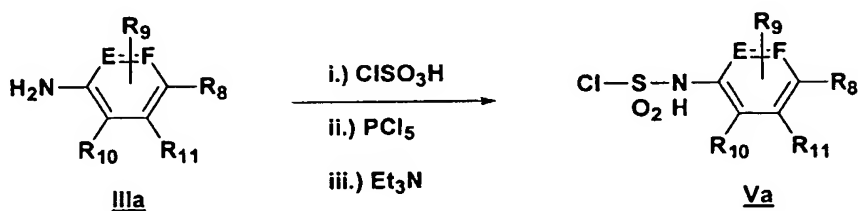
of formula **Ie** wherein  $R_2$  is an alcohol, thiol or amine. Where  $R_2$  is an alcohol, thiol or amine, compounds of formula **Ie** can be treated by methods known in the literature or readily known by one skilled in the art to yield compounds of formula **Ie** wherein  $R_2$  is a hydrogen (H),  $OR_3$ ,  $SR_3$ , halo,  $NHR_3$ ,  $NHCOR_4$ ,  $NHCO_2R_4$ ,  $NHCONR_4R_4'$  and  $NHSO_2R_4$ .

## SCHEME IV



Scheme IV describes a method to prepare sulfamoylchlorides of general formula **V** wherein intermediates **III** are treated with chlorosulfonic acid or equivalent reagent and  $\text{PCl}_5$  or equivalent reagent in the presence of an organic base such  $\text{Et}_3\text{N}$  in a solvent such dichloromethane to afford sulfamoylchlorides of formula **V**.

## SCHEME V

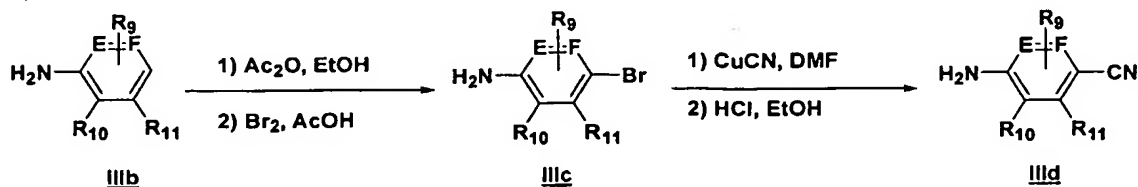


For example, Scheme V describes a method for preparing sulfamoylchlorides of general formula **Va**. Substituted aryl or heteroaryl amines of formula **IIIa** are treated with chlorosulfonic acid or equivalent reagent, and  $\text{PCl}_5$  or equivalent reagent in the presence of an organic base such  $\text{Et}_3\text{N}$  in a solvent such dichloromethane to afford sulfamoylchlorides of formula **Va**. Substituted aryl or heteroaryl amines as described above can be obtained commercially or can be prepared by methods known

in the literature or by one skilled in the art. Specific para-cyano aryl amines can be prepared according to the method outlined in Scheme VI.

### SCHEME VI

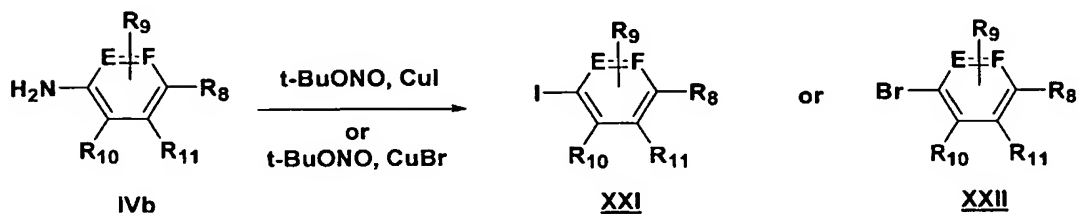
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Scheme VI describes one method for the preparation of certain cyanoaryl amines which are suitable sulfamoylchloride precursors.

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### SCHEME VII



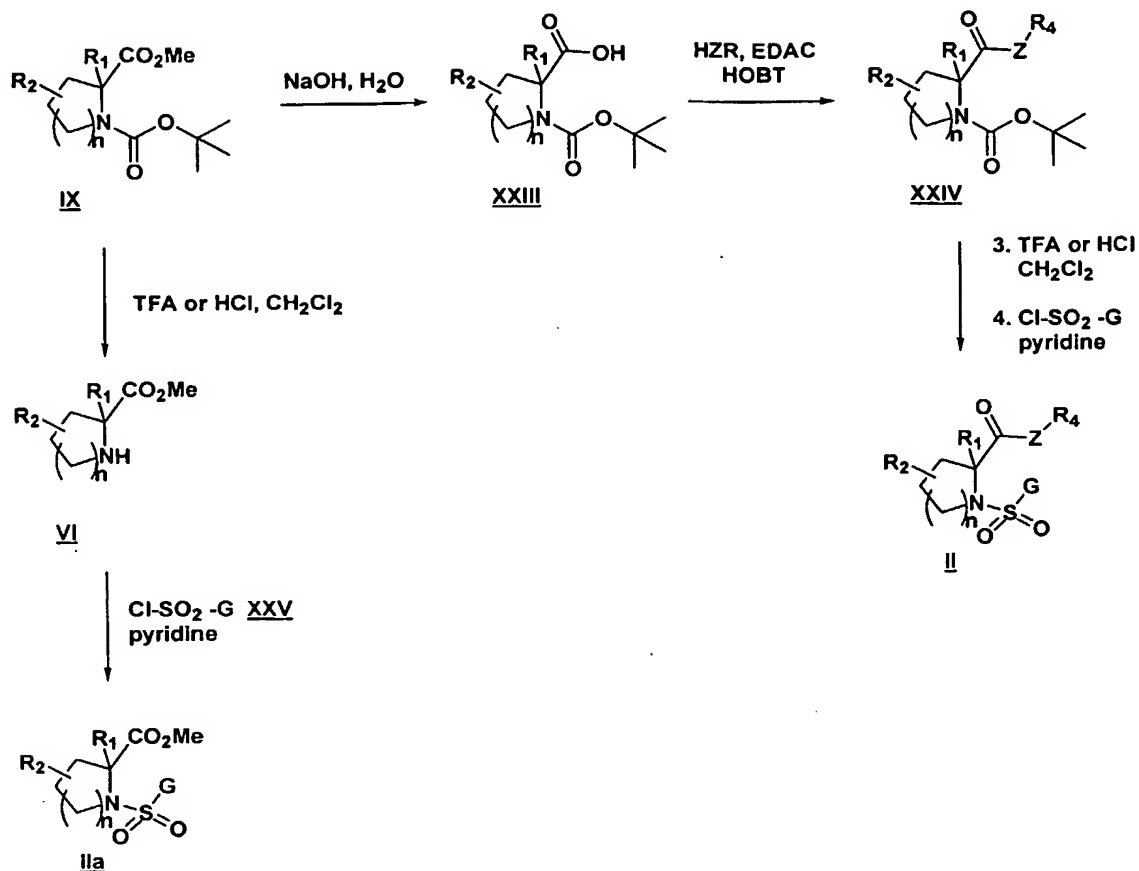
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Scheme VII describes one method for the preparation of certain aryl and heteroaryl halides precursors suitable for the formation of the intermediates of formula XIII or XVIII.

20

The chemistry used to synthesize preferred compounds of the general structure II is shown below in Scheme VIII.

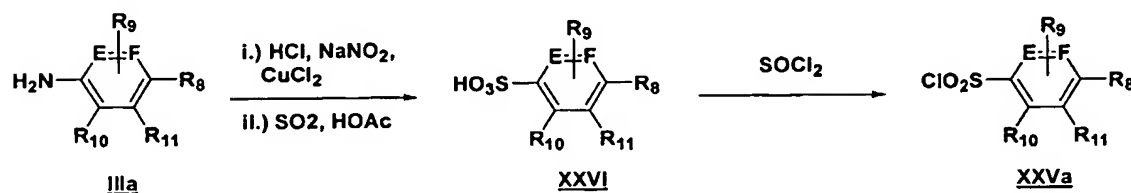
## SCHEME VIII



- 5 As illustrated in Scheme VIII, compounds of formula **II** can be prepared from intermediates **IX**. Treatment of intermediates **IX** with TFA or HCl, or equivalent reagent gives intermediates of formula **VI**. Intermediates **VI** can be treated with sulfonyl chlorides of formula  $\text{ClSO}_2\text{G}$  (**XXV**) or an equivalent agent in the presence of a base such as pyridine affords compounds of formula **IIa**. Furthermore, the
- 10 methyl ester of intermediates **IX** can be hydrolyzed with a reagent such as NaOH in a solvent such as water and/or methanol to give intermediates of formula **XXIII**. Coupling of intermediates **XXIII** to  $\text{HZR}_4$  with reagents such as EDAC and HOBT in a solvent such as dichloromethane or DMF provides intermediates of formula **XXIV**. Treatment of intermediates **XXIV** with TFA or HCl, or equivalent reagent followed
- 15 by treatment with sulfonyl chlorides of formula  $\text{ClSO}_2\text{G}$  or an equivalent agent in the presence of a base such as pyridine affords compounds of formula **II**. Where  $\text{R}_2$  is a

suitably protected alcohol, thiol or amine, compounds of formula **II** can be treated by methods known in the literature or readily known by one skilled in the art to yield compounds of formula **II** wherein  $R_2$  is an alcohol, thiol or amine. Where  $R_2$  is an alcohol, thiol or amine, compounds of formula **II** can be treated by methods known in the literature or readily known by one skilled in the art to yield compounds of formula **II** wherein  $R_2$  is a hydrogen (H),  $OR_3$ ,  $SR_3$ , halo,  $NHR_3$ ,  $NHCOR_4$ ,  $NHCO_2R_4$ ,  $NHCONR_4R_4'$  and  $NHSO_2R_4$ .

### SCHEME IX



Scheme IX describes one method for the preparation of certain sulfonylchlorides precursors suitable for the formation of compounds of formula **II**.

### USE AND UTILITY

#### A. Utilities

The compounds of the present invention modulate the function of the nuclear hormone receptors, particularly the androgen receptor, and include compounds which are, for example, selective agonists, partial agonists, antagonists or partial antagonists of the androgen receptor (AR). Thus, the present compounds are useful in the treatment of AR-associated conditions. An "AR-associated condition," as used herein, denotes a condition or disorder which can be treated by modulating the function or activity of an AR in a subject, wherein treatment comprises prevention, partial alleviation or cure of the condition or disorder. Modulation may occur locally, for example, within certain tissues of the subject, or more extensively throughout a subject being treated for such a condition or disorder.

The compounds of the present invention can be administered to animals, preferably humans, for the treatment of a variety of conditions and disorders,

including, but not limited to maintenance of muscle strength and function (e.g., in the elderly); reversal or prevention of frailty or age-related functional decline ("ARFD") in the elderly (e.g., sarcopenia); treatment of catabolic side effects of glucocorticoids; prevention and/or treatment of reduced bone mass, density or growth (e.g., osteoporosis and osteopenia); treatment of chronic fatigue syndrome (CFS); chronic myalgia; treatment of acute fatigue syndrome and muscle loss following elective surgery (e.g., post-surgical rehabilitation); accelerating of wound healing; accelerating bone fracture repair (such as accelerating the recovery of hip fracture patients); accelerating healing of complicated fractures, e.g. distraction osteogenesis; in joint replacement; prevention of post-surgical adhesion formation; acceleration of tooth repair or growth; maintenance of sensory function (e.g., hearing, sight, olfaction and taste); treatment of periodontal disease; treatment of wasting secondary to fractures and wasting in connection with chronic obstructive pulmonary disease (COPD), chronic liver disease, AIDS, weightlessness, cancer cachexia, burn and trauma recovery, chronic catabolic state (e.g., coma), eating disorders (e.g., anorexia) and chemotherapy; treatment of cardiomyopathy; treatment of thrombocytopenia; treatment of growth retardation in connection with Crohn's disease; treatment of short bowel syndrome; treatment of irritable bowel syndrome; treatment of inflammatory bowel disease; treatment of Crohn's disease and ulcerative colitis; treatment of complications associated with transplantation; treatment of physiological short stature including growth hormone deficient children and short stature associated with chronic illness; treatment of obesity and growth retardation associated with obesity; treatment of anorexia (e.g., associated with cachexia or aging); treatment of hypercortisolism and Cushing's syndrome; Paget's disease; treatment of osteoarthritis; induction of pulsatile growth hormone release; treatment of osteochondrodysplasias; treatment of depression, nervousness, irritability and stress; treatment of reduced mental energy and low self-esteem (e.g., motivation/assertiveness); improvement of cognitive function (e.g., the treatment of dementia, including Alzheimer's disease and short term memory loss); treatment of catabolism in connection with pulmonary dysfunction and ventilator dependency; treatment of cardiac dysfunction (e.g., associated with valvular disease, myocardial infarction, cardiac hypertrophy or congestive heart failure); lowering blood pressure; protection against ventricular

dysfunction or prevention of reperfusion events; treatment of adults in chronic dialysis; reversal or slowing of the catabolic state of aging; attenuation or reversal of protein catabolic responses following trauma (e.g., reversal of the catabolic state associated with surgery, congestive heart failure, cardiac myopathy, burns, cancer, COPD etc.); reducing cachexia and protein loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidioblastosis; treatment of immunosuppressed patients; treatment of wasting in connection with multiple sclerosis or other neurodegenerative disorders; promotion of myelin repair; maintenance of skin thickness; treatment of metabolic homeostasis and renal homeostasis (e.g., in the frail elderly); stimulation of osteoblasts, bone remodeling and cartilage growth; regulation of food intake; treatment of insulin resistance, including NIDDM, in mammals (e.g., humans); treatment of insulin resistance in the heart; improvement of sleep quality and correction of the relative hyposomatotropism of senescence due to high increase in REM sleep and a decrease in REM latency; treatment of hypothermia; treatment of congestive heart failure; treatment of lipodystrophy (e.g., in patients taking HIV or AIDS therapies such as protease inhibitors); treatment of muscular atrophy (e.g., due to physical inactivity, bed rest or reduced weight-bearing conditions); treatment of musculoskeletal impairment (e.g., in the elderly); improvement of the overall pulmonary function; treatment of sleep disorders; and the treatment of the catabolic state of prolonged critical illness; treatment of hirsutism, acne, seborrhea, androgenic alopecia, anemia, hyperpilosity, benign prostate hypertrophy, adenomas and neoplasies of the prostate (e.g., advanced metastatic prostate cancer) and malignant tumor cells containing the androgen receptor, such as is the case for breast, brain, skin, ovarian, bladder, lymphatic, liver and kidney cancers; cancers of the skin, pancreas, endometrium, lung and colon; osteosarcoma; hypercalcemia of malignancy; metastatic bone disease; treatment of spermatogenesis, endometriosis and polycystic ovary syndrome; counteracting preeclampsia, eclampsia of pregnancy and preterm labor; treatment of premenstrual syndrome; treatment of vaginal dryness; age related decreased testosterone levels in men, male menopause, hypogonadism, male hormone replacement, male and female sexual dysfunction (e.g., erectile dysfunction, decreased sex drive, sexual well-being, decreased libido), urinary incontinence, male and female contraception, hair loss,

Reaven's Syndrome and the enhancement of bone and muscle performance/strength. The term treatment is also intended to include prophylactic treatment.

In addition, the conditions, diseases, and maladies collectively referenced to as "Syndrome X" or Metabolic Syndrome as detailed in Johannsson *J. Clin. Endocrinol. Metab.*, 82, 727-34 (1997), may be treated employing the compounds of the invention.

#### B. Combinations

The present invention includes within its scope pharmaceutical compositions comprising, as an active ingredient, a therapeutically effective amount of at least one of the compounds of formula I, alone or in combination with a pharmaceutical carrier or diluent. Optionally, compounds of the present invention can be used alone, in combination with other compounds of the invention, or in combination with one or more other therapeutic agent(s), e.g., an antibiotic or other pharmaceutically active material.

The compounds of the present invention may be combined with growth promoting agents, such as, but not limited to, TRH, diethylstilbesterol, theophylline, enkephalins, E series prostaglandins, compounds disclosed in U.S. Patent No. 3,239,345, e.g., zeranol, and compounds disclosed in U.S. Patent No. 4,036,979, e.g., sulbenox or peptides disclosed in U.S. Patent No. 4,411,890.

The compounds of the invention may also be used in combination with growth hormone secretagogues such as GHRP-6, GHRP-1 (as described in U.S. Patent No. 4,411,890 and publications WO 89/07110 and WO 89/07111), GHRP-2 (as described in WO 93/04081), NN703 (Novo Nordisk), LY444711 (Lilly), MK-677 (Merck), CP424391 (Pfizer) and B-HT920, or with growth hormone releasing factor and its analogs or growth hormone and its analogs or somatomedins including IGF-1 and IGF-2, or with alpha-adrenergic agonists, such as clonidine or serotonin 5-HT<sub>D</sub> agonists, such as sumatriptan, or agents which inhibit somatostatin or its release, such as physostigmine and pyridostigmine. A still further use of the disclosed compounds of the invention is in combination with parathyroid hormone, PTH(1-34) or bisphosphonates, such as MK-217 (alendronate).

A still further use of the compounds of the invention is in combination with estrogen, testosterone, a selective estrogen receptor modulator, such as tamoxifen or



raloxifene, or other androgen receptor modulators, such as those disclosed in Edwards, J. P. *et. al.*, *Bio. Med. Chem. Let.*, 9, 1003-1008 (1999) and Hamann, L. G. *et. al.*, *J. Med. Chem.*, 42, 210-212 (1999).

5 A further use of the compounds of this invention is in combination with progesterone receptor agonists ("PRA"), such as levonorgestrel, medroxyprogesterone acetate (MPA).

The compounds of the present invention may be employed alone or in combination with each other and/or other modulators of nuclear hormone receptors or other suitable therapeutic agents useful in the treatment of the aforementioned disorders including: anti-diabetic agents; anti-osteoporosis agents; anti-obesity  
10 agents; anti-inflammatory agents; anti-anxiety agents; anti-depressants; anti-hypertensive agents; anti-platelet agents; anti-thrombotic and thrombolytic agents; cardiac glycosides; cholesterol/lipid lowering agents; mineralocorticoid receptor antagonists; phosphodiesterase inhibitors; protein tyrosine kinase inhibitors; thyroid  
15 mimetics (including thyroid receptor agonists); anabolic agents; HIV or AIDS therapies; therapies useful in the treatment of Alzheimer's disease and other cognitive disorders; therapies useful in the treatment of sleeping disorders; anti-proliferative agents; and anti-tumor agents.

Examples of suitable anti-diabetic agents for use in combination with the  
20 compounds of the present invention include biguanides (e.g., metformin), glucosidase inhibitors (e.g., acarbose), insulins (including insulin secretagogues or insulin sensitizers), meglitinides (e.g., repaglinide), sulfonylureas (e.g., glimepiride, glyburide and glipizide), biguanide/glyburide combinations (e.g., Glucovance<sup>®</sup>), thiazolidinediones (e.g., troglitazone, rosiglitazone and pioglitazone), PPAR-alpha  
25 agonists, PPAR-gamma agonists, PPAR alpha/gamma dual agonists, SGLT2 inhibitors, glycogen phosphorylase inhibitors, inhibitors of fatty acid binding protein (aP2) such as those disclosed in U.S. Serial No. 09/519,079 filed March 6, 2000, glucagon-like peptide-1 (GLP-1), and dipeptidyl peptidase IV (DPP4) inhibitors such as those disclosed in WO 0168603.

30 Examples of suitable anti-osteoporosis agents for use in combination with the compounds of the present invention include alendronate, risedronate, PTH, PTH fragment, raloxifene, calcitonins, steroidal or non-steroidal progesterone receptor

agonists, RANK ligand antagonists, calcium sensing receptor antagonists, TRAP inhibitors, selective estrogen receptor modulators (SERM's), estrogen and AP-1 inhibitors.

Examples of suitable anti-obesity agents for use in combination with the  
5 compounds of the present invention include aP2 inhibitors, such as those disclosed in U.S. Serial No. 09/519,079 filed March 6, 2000, PPAR gamma antagonists, PPAR delta agonists, beta 3 adrenergic agonists, such as AJ9677 (Takeda/Dainippon), L750355 (Merck), or CP331648 (Pfizer) or other known beta 3 agonists as disclosed in U.S. Patent Nos. 5,541,204, 5,770,615, 5,491,134, 5,776,983 and 5,488,064, a  
10 lipase inhibitor, such as orlistat or ATL-962 (Alizyme), a serotonin (and dopamine) reuptake inhibitor, such as sibutramine, topiramate (Johnson & Johnson) or axokine (Regeneron), a thyroid receptor beta drug, such as a thyroid receptor ligand as disclosed in WO 97/21993 (U. Cal SF), WO 99/00353 (KaroBio) and GB98/284425 (KaroBio), and/or an anorectic agent, such as dexamphetamine, phentermine,  
15 phenylpropanolamine or mazindol.

Examples of suitable anti-inflammatory agents for use in combination with the compounds of the present invention include prednisone, dexamethasone, Enbrel<sup>®</sup>, cyclooxygenase inhibitors (i.e., COX-1 and/or COX-2 inhibitors such as NSAIDs, aspirin, indomethacin, ibuprofen, piroxicam, Naproxen<sup>®</sup>, Celebrex<sup>®</sup>, Vioxx<sup>®</sup>),  
20 CTLA4-Ig agonists/antagonists, CD40 ligand antagonists, IMPDH inhibitors, such as mycophenolate (CellCept<sup>®</sup>), integrin antagonists, alpha-4 beta-7 integrin antagonists, cell adhesion inhibitors, interferon gamma antagonists, ICAM-1, tumor necrosis factor (TNF) antagonists (e.g., infliximab, OR1384), prostaglandin synthesis inhibitors, budesonide, clofazimine, CNI-1493, CD4 antagonists (e.g., priliximab),  
25 p38 mitogen-activated protein kinase inhibitors, protein tyrosine kinase (PTK) inhibitors, IKK inhibitors, and therapies for the treatment of irritable bowel syndrome (e.g., Zelmac<sup>®</sup> and Maxi-K<sup>®</sup> openers such as those disclosed in U.S. Patent No. 6,184,231 B1).

Examples of suitable anti-anxiety agents for use in combination with the  
30 compounds of the present invention include diazepam, lorazepam, buspirone, oxazepam, and hydroxyzine pamoate.

Examples of suitable anti-depressants for use in combination with the compounds of the present invention include citalopram, fluoxetine, nefazodone, sertraline, and paroxetine.

5 Examples of suitable anti-hypertensive agents for use in combination with the compounds of the present invention include beta adrenergic blockers, calcium channel blockers (L-type and T-type; e.g. diltiazem, verapamil, nifedipine, amlodipine and mybefradil), diuretics (e.g., chlorothiazide, hydrochlorothiazide, flumethiazide, hydroflumethiazide, bendroflumethiazide, methylchlorothiazide, trichloromethiazide, polythiazide, benzthiazide, ethacrynic acid tricrynafen, chlorthalidone, furosemide, 10 musolimine, bumetanide, triamtrenene, amiloride, spironolactone), renin inhibitors, ACE inhibitors (e.g., captopril, zofenopril, fosinopril, enalapril, ceranopril, cilazopril, delapril, pentopril, quinapril, ramipril, lisinopril), AT-1 receptor antagonists (e.g., losartan, irbesartan, valsartan), ET receptor antagonists (e.g., sitaxsentan, atrsentan and compounds disclosed in U.S. Patent Nos. 5,612,359 and 6,043,265), Dual ET/AII 15 antagonist (e.g., compounds disclosed in WO 00/01389), neutral endopeptidase (NEP) inhibitors, vasopepsidase inhibitors (dual NEP-ACE inhibitors) (e.g., omapatrilat and gemopatrilat), and nitrates.

Examples of suitable anti-platelet agents for use in combination with the compounds of the present invention include GPIIb/IIIa blockers (e.g., abciximab, 20 eptifibatide, tirofiban), P2Y<sub>12</sub> antagonists (e.g., clopidogrel, ticlopidine, CS-747), thromboxane receptor antagonists (e.g., ifetroban), aspirin, and PDE-III inhibitors (e.g., dipyridamole) with or without aspirin.

Examples of suitable cardiac glycosides for use in combination with the compounds of the present invention include digitalis and ouabain.

25 Examples of suitable cholesterol/lipid lowering agents for use in combination with the compounds of the present invention include HMG-CoA reductase inhibitors (e.g., pravastatin, lovastatin, atorvastatin, simvastatin, NK-104 (a.k.a. itavastatin, or nisvastatin or nisbastatin) and ZD-4522 (a.k.a. rosuvastatin, or atavastatin or visastatin)), squalene synthetase inhibitors, fibrates, bile acid sequestrants, ACAT 30 inhibitors, MTP inhibitors, lipooxygenase inhibitors, cholesterol absorption inhibitors, and cholesterol ester transfer protein inhibitors (e.g., CP-529414).

Examples of suitable mineralocorticoid receptor antagonists for use in combination with the compounds of the present invention include spironolactone and eplerinone.

5        Examples of suitable phosphodiesterase (PDE) inhibitors for use in combination with the compounds of the present invention include PDE-3 inhibitors such as cilostazol, and phosphodiesterase-5 inhibitors (PDE-5 inhibitors) such as sildenafil.

10       Examples of suitable thyroid mimetics for use in combination with the compounds of the present invention include thyrotropin, polythyroid, KB-130015, and dronedarone.

15       Examples of suitable anabolic agents for use in combination with the compounds of the present invention include testosterone, TRH diethylstilbesterol, estrogens,  $\beta$ -agonists, theophylline, anabolic steroids, dehydroepiandrosterone, enkephalins, E-series prostagladins, retinoic acid and compounds as disclosed in U.S. Pat. No. 3,239,345, e.g., Zeranol<sup>®</sup>; U.S. Patent No. 4,036,979, e.g., Sulbenox<sup>®</sup> or peptides as disclosed in U.S. Pat. No. 4,411,890.

20       Examples of suitable HIV or AIDS therapies for use in combination with the compounds of the present invention include indinavir sulfate, saquinavir, saquinavir mesylate, ritonavir, lamivudine, zidovudine, lamivudine/zidovudine combinations, zalcitabine, didanosine, stavudine, and megestrol acetate.

25       Examples of suitable therapies for treatment of Alzheimer's disease and cognitive disorders for use in combination with the compounds of the present invention include donepezil, tacrine, revastigmine, 5HT<sub>6</sub>, gamma secretase inhibitors, beta secretase inhibitors, SK channel blockers, Maxi-K blockers, and KCNQs blockers.

30       Examples of suitable therapies for treatment of sleeping disorders for use in combination with the compounds of the present invention include melatonin analogs, melatonin receptor antagonists, ML1B agonists, and GABA/NMDA receptor antagonists.

      Examples of suitable anti-proliferative agents for use in combination with the compounds of the present invention include cyclosporin A, paclitaxel, FK-506, and adriamycin.

Examples of suitable anti-tumor agents for use in combination with the compounds of the present invention include paclitaxel, adriamycin, epothilones, cisplatin and carboplatin.

Compounds of the present invention may further be used in combination with  
5 nutritional supplements such as those described in U.S. 5,179,080, especially in combination with whey protein or casein, amino acids (such as leucine, branched amino acids and hydroxymethylbutyrate), triglycerides, vitamins (e.g., A, B6, B12, folate, C, D and E), minerals (e.g., selenium, magnesium, zinc, chromium, calcium and potassium), carnitine, lipoic acid, creatinine, B-hydroxy-B-methylbutyrate (Juven)  
10 and coenzyme Q-10.

In addition, compounds of the present invention may be used in combination with therapeutic agents used in the treatment of sexual dysfunction, including but not limited to PDE-5 inhibitors, such as sildenafil or IC-351.

Compounds of the present invention may further be used in combination with  
15 antiresorptive agents, hormone replacement therapies, vitamin D analogues, elemental calcium and calcium supplements, cathepsin K inhibitors, MMP inhibitors, vitronectin receptor antagonists, Src SH<sub>2</sub> antagonists, vacuolar H<sup>+</sup>-ATPase inhibitors, ipriflavone, fluoride, Tibolone, prostanoids, 17-beta hydroxysteroid dehydrogenase inhibitors and Src kinase inhibitors.

Compounds of the present invention may be used in combination with male  
20 contraceptives, such as nonoxynol 9 or therapeutic agents for the treatment of hair loss, such as minoxidil and finasteride or chemotherapeutic agents, such as with LHRH agonists.

Further, the compounds of the present invention may be used in combination  
25 with anti-cancer and cytotoxic agents, including but not limited to alkylating agents such as nitrogen mustards, alkyl sulfonates, nitrosoureas, ethylenimines, and triazines; antimetabolites such as folate antagonists, purine analogues, and pyrimidine analogues; antibiotics such as anthracyclines, bleomycins, mitomycin, dactinomycin, and plicamycin; enzymes such as L-asparaginase; farnesyl-protein transferase  
30 inhibitors; 5 $\alpha$ -reductase inhibitors; inhibitors of 17 $\beta$ -hydroxysteroid dehydrogenase type 3; hormonal agents such as glucocorticoids, estrogens/ antiestrogens, androgens/ antiandrogens, progestins, and luteinizing hormone-releasing hormone antagonists,

octreotide acetate; microtubule-disruptor agents, such as ecteinascidins or their analogs and derivatives; microtubule-stabilizing agents such as taxanes, for example, paclitaxel (Taxol<sup>®</sup>), docetaxel (Taxotere<sup>®</sup>), and their analogs, and epothilones, such as epothilones A-F and their analogs; plant-derived products, such as vinca alkaloids, epipodophyllotoxins, taxanes; and topoisomerase inhibitors; prenyl-protein transferase inhibitors; and miscellaneous agents such as hydroxyurea, procarbazine, mitotane, hexamethylmelamine, platinum coordination complexes such as cisplatin and carboplatin; and other agents used as anti-cancer and cytotoxic agents such as biological response modifiers, growth factors; immune modulators and monoclonal antibodies. The compounds of the invention may also be used in conjunction with radiation therapy.

Representative examples of these classes of anti-cancer and cytotoxic agents include but are not limited to mechlorethamine hydrochloride, cyclophosphamide, chlorambucil, melphalan, ifosfamide, busulfan, carmustin, lomustine, semustine, streptozocin, thiotepea, dacarbazine, methotrexate, thioguanine, mercaptopurine, fludarabine, pentastatin, cladribin, cytarabine, fluorouracil, doxorubicin hydrochloride, daunorubicin, idarubicin, bleomycin sulfate, mitomycin C, actinomycin D, safracins, saframycins, quinocarcins, discodermolides, vincristine, vinblastine, vinorelbine tartrate, etoposide, etoposide phosphate, teniposide, paclitaxel, tamoxifen, estramustine, estramustine phosphate sodium, flutamide, buserelin, leuprolide, pteridines, diynes, levamisole, aflacon, interferon, interleukins, aldesleukin, filgrastim, sargramostim, rituximab, BCG, tretinoin, irinotecan hydrochloride, betamethosone, gemcitabine hydrochloride, altretamine, and topotecan and any analogs or derivatives thereof.

Preferred member of these classes include, but are not limited to, paclitaxel, cisplatin, carboplatin, doxorubicin, carminomycin, daunorubicin, aminopterin, methotrexate, methopterin, mitomycin C, ecteinascidin 743, or porfiromycin, 5-fluorouracil, 6-mercaptopurine, gemcitabine, cytosine arabinoside, podophyllotoxin or podophyllotoxin derivatives such as etoposide, etoposide phosphate or teniposide, melphalan, vinblastine, vincristine, leurosine, vindesine and leurosine.

Examples of anticancer and other cytotoxic agents include the following: epothilone derivatives as found in German Patent No. 4138042.8; WO 97/19086, WO

98/22461, WO 98/25929, WO 98/38192, WO 99/01124, WO 99/02224, WO  
99/02514, WO 99/03848, WO 99/07692, WO 99/27890, WO 99/28324, WO  
99/43653, WO 99/54330, WO 99/54318, WO 99/54319, WO 99/65913, WO  
99/67252, WO 99/67253 and WO 00/00485; cyclin dependent kinase inhibitors as  
5 found in WO 99/24416 (see also U.S. Patent No. 6,040,321); and prenyl-protein  
transferase inhibitors as found in WO 97/30992 and WO 98/54966; and agents such  
as those described generically and specifically in U.S. Patent No. 6,011,029 (the  
compounds of which U.S. Patent can be employed together with any NHR modulators  
(including, but not limited to, those of present invention) such as AR modulators, ER  
10 modulators, with LHRH modulators, or with surgical castration, especially in the  
treatment of cancer).

The above other therapeutic agents, when employed in combination with the  
compounds of the present invention, may be used, for example, in those amounts  
indicated in the Physicians' Desk Reference (PDR) or as otherwise determined by one  
15 of ordinary skill in the art.

The compounds of the formula I can be administered for any of the uses  
described herein by any suitable means, for example, orally, such as in the form of  
tablets, capsules, granules or powders; sublingually; buccally; parenterally, such as by  
subcutaneous, intravenous, intramuscular, or intrasternal injection or infusion  
20 techniques (e.g., as sterile injectable aqueous or non-aqueous solutions or  
suspensions); nasally, including administration to the nasal membranes, such as by  
inhalation spray; topically, such as in the form of a cream or ointment; or rectally such  
as in the form of suppositories; in dosage unit formulations containing non-toxic,  
pharmaceutically acceptable vehicles or diluents. The present compounds can, for  
25 example, be administered in a form suitable for immediate release or extended  
release. Immediate release or extended release can be achieved by the use of suitable  
pharmaceutical compositions comprising the present compounds, or, particularly in  
the case of extended release, by the use of devices such as subcutaneous implants or  
osmotic pumps. The present compounds can also be administered liposomally.

30 Exemplary compositions for oral administration include suspensions which  
can contain, for example, microcrystalline cellulose for imparting bulk, alginic acid or  
sodium alginate as a suspending agent, methylcellulose as a viscosity enhancer, and

sweeteners or flavoring agents such as those known in the art; and immediate release tablets which can contain, for example, microcrystalline cellulose, dicalcium phosphate, starch, magnesium stearate and/or lactose and/or other excipients, binders, extenders, disintegrants, diluents and lubricants such as those known in the art. The compounds of formula I can also be delivered through the oral cavity by sublingual and/or buccal administration. Molded tablets, compressed tablets or freeze-dried tablets are exemplary forms which may be used. Exemplary compositions include those formulating the present compound(s) with fast dissolving diluents such as mannitol, lactose, sucrose and/or cyclodextrins. Also included in such formulations may be high molecular weight excipients such as celluloses (avicel) or polyethylene glycols (PEG). Such formulations can also include an excipient to aid mucosal adhesion such as hydroxy propyl cellulose (HPC), hydroxy propyl methyl cellulose (HPMC), sodium carboxy methyl cellulose (SCMC), maleic anhydride copolymer (e.g., Gantrez), and agents to control release such as polyacrylic copolymer (e.g. Carbopol 934). Lubricants, glidants, flavors, coloring agents and stabilizers may also be added for ease of fabrication and use.

Exemplary compositions for nasal aerosol or inhalation administration include solutions in saline which can contain, for example, benzyl alcohol or other suitable preservatives, absorption promoters to enhance bioavailability, and/or other solubilizing or dispersing agents such as those known in the art.

Exemplary compositions for parenteral administration include injectable solutions or suspensions which can contain, for example, suitable non-toxic, parenterally acceptable diluents or solvents, such as mannitol, 1,3-butanediol, water, Ringer's solution, an isotonic sodium chloride solution, or other suitable dispersing or wetting and suspending agents, including synthetic mono- or diglycerides, and fatty acids, including oleic acid, or Cremaphor.

Exemplary compositions for rectal administration include suppositories which can contain, for example, a suitable non-irritating excipient, such as cocoa butter, synthetic glyceride esters or polyethylene glycols, which are solid at ordinary temperatures, but liquify and/or dissolve in the rectal cavity to release the drug.

Exemplary compositions for topical administration include a topical carrier such as Plastibase (mineral oil gelled with polyethylene).



The effective amount of a compound of the present invention can be determined by one of ordinary skill in the art, and includes exemplary dosage amounts for an adult human of from about 0.01 to 2000 mg of active compound per day, which can be administered in a single dose or in the form of individual divided doses, such as from 1 to 4 times per day. It will be understood that the specific dose level and frequency of dosage for any particular subject can be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the species, age, body weight, general health, sex and diet of the subject, the mode and time of administration, rate of excretion, drug combination, and severity of the particular condition. Preferred subjects for treatment include animals, most preferably mammalian species such as humans, and domestic animals such as dogs, cats and the like, subject to NHR-associated conditions.

15

## TRANSACTIVATION ASSAYS

### AR Specific Assay

Compounds of the present invention were tested in transactivation assays of a transfected reporter construct and using the endogenous androgen receptor of the host cells. The transactivation assay provides a method for identifying functional agonists and partial agonists that mimic, or antagonists that inhibit, the effect of native hormones, in this case, dihydrotestosterone (DHT). This assay can be used to predict *in vivo* activity as there is a good correlation in both series of data. See, e.g. T. Berger et al., *J. Steroid Biochem. Molec. Biol.* 773 (1992), the disclosure of which is herein incorporated by reference.

For the transactivation assay a reporter plasmid is introduced by transfection (a procedure to induce cells to take foreign genes) into the respective cells. This reporter plasmid, comprising the cDNA for a reporter protein, such as secreted alkaline phosphatase (SEAP), controlled by prostate specific antigen (PSA) upstream sequences containing androgen response elements (AREs). This reporter plasmid functions as a reporter for the transcription-modulating activity of the AR. Thus, the reporter acts as a surrogate for the products (mRNA then protein) normally expressed by a gene under control of the AR and its native hormone. In order to detect

antagonists, the transactivation assay is carried out in the presence of constant concentration of the natural AR hormone (DHT) known to induce a defined reporter signal. Increasing concentrations of a suspected antagonist will decrease the reporter signal (e.g., SEAP production). On the other hand, exposing the transfected cells to  
5 increasing concentrations of a suspected agonist will increase the production of the reporter signal.

For this assay, LNCaP and MDA 453 cells were obtained from the American Type Culture Collection (Rockville, MD), and maintained in RPMI 1640 or DMEM medium supplemented with 10% fetal bovine serum (FBS; Gibco) respectively. The  
10 respective cells were transiently transfected by electroporation according to the optimized procedure described by Heiser, 130 Methods Mol. Biol., 117 (2000), with the pSEAP2/PSA540/Enhancer reporter plasmid. The reporter plasmid, was constructed as follows: commercial human placental genomic DNA was used to generate by Polymerase Cycle Reaction (PCR) a fragment containing the BglII site  
15 (position 5284) and the Hind III site at position 5831 of the human prostate specific antigen promoter (Accession # U37672), Schuur, et al., *J. Biol. Chem.*, 271 (12): 7043-51 (1996). This fragment was subcloned into the pSEAP2/basic (Clontech) previously digested with BglII and HindIII to generate the pSEAP2/PSA540 construct. Then a fragment bearing the fragment of human PSA upstream sequence between  
20 positions -5322 and -3873 was amplified by PCR from human placental genomic DNA. A XhoI and a BglII sites were introduced with the primers. The resulting fragment was subcloned into pSEAP2/PSA540 digested with XhoI and BglII respectively, to generate the pSEAP2/PSA540/Enhancer construct. LNCaP and MDA MB-453 cells were collected in media containing 10% charcoal stripped FBS. Each  
25 cell suspension was distributed into two Gene Pulser Cuvetts (Bio-Rad) which then received 8 µg of the reporter construct, and electroporated using a Bio-Rad Gene Pulser at 210 volts and 960 µFaraday. Following the transfections the cells were washed and incubated with media containing charcoal stripped fetal bovine serum in the absence (blank) or presence (control) of 1 nM dihydrotestosterone (DHT; Sigma  
30 Chemical) and in the presence or absence of the standard anti-androgen bicalutamide or compounds of the present invention in concentrations ranging from  $10^{-10}$  to  $10^{-5}$  M

(sample). Duplicates were used for each sample. The compound dilutions were performed on a Biomek 2000 laboratory workstation.

After 48 h, a fraction of the supernatant was assayed for SEAP activity using the Phospha-Light Chemiluminescent Reporter Gene Assay System (Tropix, Inc).

- 5 Viability of the remaining cells was determined using the CellTiter 96 Aqueous Non-Radioactive Cell Proliferation Assay (MTS Assay, Promega). Briefly, a mix of a tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)- 2-(4-sulphophenyl)-2H-tetrazolium, inner salt; MTS) and an electron coupling reagent (phenazine methosulfate; PMS) are added to the cells. MTS (Owen's reagent) is
- 10 bioreduced by cells into a formazan that is soluble in tissue culture medium, and therefore its absorbance at 490 nm can be measured directly from 96 well assay plates without additional processing. The quantity of formazan product as measured by the amount of 490 nm absorbance is directly proportional to the number of living cells in culture. For each replicate the SEAP reading was normalized by the Abs490 value
- 15 derived from the MTS assay. For the antagonist mode, the % Inhibition was calculated as:

% Inhibition =  $100 \times (1 - [\text{average control} - \text{average blank} / \text{average sample} - \text{average blank}])$

20

Data was plotted and the concentration of compound that inhibited 50% of the normalized SEAP was quantified ( $IC_{50}$ ).

- 25 For the agonist mode % Control was referred as the effect of the tested compound compared to the maximal effect observed with the natural hormone, in this case DHT, and was calculated as:

% Control =  $100 \times \text{average sample} - \text{average blank} / \text{average control} - \text{average blank}$

- 30 Data was plotted and the concentration of compound that activates to levels 50% of the normalized SEAP for the control was quantified ( $EC_{50}$ ).

### GR Specificity Assay

The reporter plasmid utilized was comprised of the cDNA for the reporter SEAP protein, as described for the AR specific transactivation assay. Expression of the reporter SEAP protein was controlled by the mouse mammary tumor virus long terminal repeat (MMTV LTR) sequences that contains three hormone response elements (HREs) that can be regulated by both GR and PR see, e.g. G. Chalepakis et al., Cell, 53(3), 371 (1988). This plasmid was transfected into A549 cells, which expresses endogenous GR, to obtain a GR specific transactivation assay. A549 cells were obtained from the American Type Culture Collection (Rockville, MD), and maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS; Gibco). Determination of the GR specific antagonist activity of the compounds of the present invention was identical to that described for the AR specific transactivation assay, except that the DHT was replaced with 5 nM dexamethasone (Sigma Chemicals), a specific agonist for GR. Determination of the GR specific agonist activity of the compounds of the present invention was performed as described for the AR transactivation assay, wherein one measures the activation of the GR specific reporter system by the addition of a test compound, in the absence of a known GR specific agonists ligand.

### PR Specific Assay

The reporter plasmid utilized was comprised of the cDNA for the reporter SEAP protein, as described for the AR specific transactivation assay. Expression of the reporter SEAP protein was controlled by the mouse mammary tumor virus long terminal repeat (MMTV LTR) sequences that contains three hormone response elements (HRE's) that can be regulated by both GR and PR. This plasmid was transfected into T47D, which expresses endogenous PR, to obtain a PR specific transactivation assay. T47D cells were obtained from the American Type Culture Collection (Rockville, MD), and maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS; Gibco). Determination of the PR specific antagonist activity of the compounds of the present invention was identical to that described for the AR specific transactivation assay, except that the DHT was replaced with 1 nM Promegastone (NEN), a specific agonist for PR. Determination of the PR specific

agonist activity of the compounds of the present invention was performed as described for the AR transactivation assay, wherein one measures the activation of the PR specific reporter system by the addition of a test compound, in the absence of a known PR specific agonists ligand.

5

### AR Binding Assay

For the whole-cell binding assay, human LNCaP cells (T877A mutant AR) or MDA 453 (wild type AR) in 96-well microtiter plates containing RPMI 1640 or DMEM supplemented with 10% charcoal stripped CA-FBS (Cocaleco Biologicals) respectively, were incubated at 37 °C to remove any endogenous ligand that might be complexed with the receptor in the cells. After 48 h, either a saturation analysis to determine the  $K_d$  for tritiated dihydrotestosterone, [ $^3$ H]-DHT, or a competitive binding assay to evaluate the ability of test compounds to compete with [ $^3$ H]-DHT were performed. For the saturation analysis, media (RPMI 1640 or DMEM – 0.2% CA-FBS) containing [ $^3$ H]-DHT (in concentrations ranging from 0.1 nM to 16 nM) in the absence (total binding) or presence (non-specific binding) of a 500-fold molar excess of unlabeled DHT were added to the cells. After 4 h at 37 °C, an aliquot of the total binding media at each concentration of [ $^3$ H]-DHT was removed to estimate the amount of free [ $^3$ H]-DHT. The remaining media was removed, cells were washed  
15  
20 three times with PBS and harvested onto UniFilter GF/B plates (Packard), Microscint (Packard) was added and plates counted in a Top-Counter (Packard) to evaluate the amount of bound [ $^3$ H]-DHT.

For the saturation analysis, the difference between the total binding and the non-specific binding, was defined as specific binding. The specific binding was  
25 evaluated by Scatchard analysis to determine the  $K_d$  for [ $^3$ H]-DHT. See e.g. D. Rodbard, Mathematics and statistics of ligand assays: an illustrated guide: In: J. Langon and J. J. Clapp, eds., Ligand Assay, Masson Publishing U.S.A., Inc., New York, pp. 45-99, (1981), the disclosure of which is herein incorporated by reference.

For the competition studies, media containing 1 nM [ $^3$ H]-DHT and  
30 compounds of the invention ("test compounds") in concentrations ranging from  $10^{-10}$  to  $10^{-5}$  M were added to the cells. Two replicates were used for each sample. After 4 h at 37 °C, cells were washed, harvested and counted as described above. The data

was plotted as the amount of [<sup>3</sup>H]-DHT (% of control in the absence of test compound) remaining over the range of the dose response curve for a given compound. The concentration of test compound that inhibited 50% of the amount of [<sup>3</sup>H]-DHT bound in the absence of competing ligand was quantified (IC<sub>50</sub>) after log-  
5 logit transformation. The K<sub>i</sub> values were determined by application of the Cheng-Prusoff equation to the IC<sub>50</sub> values, where:

$$K_i = \frac{IC_{50}}{(1 + ([^3H\text{-DHT}] / K_d \text{ for } ^3H\text{-DHT}))}$$

10

After correcting for non-specific binding, IC<sub>50</sub> values were determined. The IC<sub>50</sub> is defined as the concentration of competing ligand needed to reduce specific binding by 50%. The K<sub>d</sub>s for [<sup>3</sup>H]-DHT for MDA 453 and LNCaP were 0.7 and 0.2 nM respectively.

15

#### **C2C12 Mouse Myoblast Transactivation Assay**

Two functional transactivation assays were developed to assess the efficacy of androgen agonists in a muscle cell background using a luciferase reporter. The first assay (ARTA Stable 1) uses a cell line, Stable 1 (clone #72), which expresses the full  
20 length rat androgen receptor but requires the transient transfection of an enhancer/reporter. This cell line was derived from C2C12 mouse myoblast cells. The second assay (ARTA Stable 2) uses a cell line, Stable 2 (clone #133), derived from Stable 1 which expresses both rAR and the enhancer/luciferase reporter.

The enhancer/reporter construct used in this system is pGL3/2XDR-  
25 1/luciferase. 2XDR-1 was reported to be an AR specific response element in CV-1 cells, Brown et. al. The Journal of Biological Chemistry 272, 8227-8235, (1997). It was developed by random mutagenesis of an AR/GR consensus enhancer sequence.

#### **ARTA Stable 1**

30 1. Stable 1 cells are plated in 96 well format at 6,000 cells/well in high glucose DMEM without phenol red (Gibco BRL, Cat. No.: 21063-029) containing 10% charcoal and dextran treated FBS (HyClone Cat. No.: SH30068.02), 50 mM

HEPES Buffer (Gibco BRL, Cat. No.: 15630-080), 1X MEM Na Pyruvate (Gibco BRL, Cat. No.: 11360-070), 0.5X Antibiotic-Antimycotic, and 800 µg/mL Geneticin (Gibco BRL, Cat. No.: 10131-035).

2. 48 h later, cells are transfected with pGL3/2XDR-1/luciferase using  
5 LipofectAMINE Plus<sup>TM</sup> Reagent (Gibco BRL, Cat. No.: 10964-013). Specifically, 5 ng/well pGL3/2XDR-1/luciferase DNA and 50 ng/well Salmon Sperm DNA (as carrier) are diluted with 5 µl/well Opti-MEM media (Gibco BRL, Cat. No.: 31985-070). To this, 0.5 µl/well Plus reagent is added. This mixture is incubated for 15 min at rt. In a separate vessel, 0.385 µl/well LipofectAMINE reagent is diluted with 5  
10 µl/well Opti-MEM. The DNA mixture is then combined with the LipofectAMINE mixture and incubated for an additional 15 min at rt. During this time, the media from the cells is removed and replaced with 60 µl/well of Opti-MEM. To this is added 10 µl/well of the DNA/LipofectAMINE transfection mixture. The cells are incubated for 4 h.
- 15 3. The transfection mixture is removed from the cells and replaced with 90 µl of media as in #1 above.
4. 10 µl/well of appropriate drug dilution is placed in each well.
5. 24 h later, the Steady-Glo<sup>TM</sup> Luciferase Assay System is used to detect activity according to the manufacturer's instructions (Promega, Cat. No.: E2520).

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#### **ARTA Stable 2**

1. Stable 2 cells are plated in 96 well format at 6,000 cells/well in high glucose DMEM without phenol red (Gibco BRL, Cat. No.: 21063-029) containing 10% charcoal and dextran treated FBS (HyClone Cat. No.: SH30068.02), 50 mM  
25 HEPES Buffer (Gibco BRL, Cat. No.: 15630-080), 1X MEM Na Pyruvate (Gibco BRL, Cat. No.: 11360-070), 0.5X Antibiotic-Antimycotic, 800 µg/mL Geneticin (Gibco BRL, Cat. No.: 10131-035) and 800 µg/mL Hygromycin β (Gibco BRL, Cat. No.: 10687-010).
2. 48 h later, the media on the cells is removed and replaced with 90 µl  
30 fresh. 10 µl/well of appropriate drug dilution is placed in each well.

3. 24 h later, the Steady-Glo™ Luciferase Assay System is used to detect activity according to the manufacturer's instructions (Promega, Cat. No. E2520).

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## PROLIFERATION ASSAYS

### Human Prostate Cell Proliferation Assay

Compounds of the present invention were tested ("test compounds") on the proliferation of human prostate cancer cell lines. For that, MDA PCa2b cells, a cell line derived from the metastasis of a patient that failed castration, Navone et al., Clin. Cancer Res., 3, 2493-500 (1997), were incubated with or without the test compounds for 72 h and the amount of [<sup>3</sup>H]-thymidine incorporated into DNA was quantified as a way to assess number of cells and therefore proliferation. The MDA PCa2b cell line was maintained in BRFF-HPC1 media (Biological Research Faculty & Facility Inc., MD) supplemented with 10% FBS. For the assay, cells were plated in Biocoated 96-well microplates and incubated at 37 °C in 10% FBS (charcoal-stripped)/BRFF-BMZERO (without androgens). After 24 h, the cells were treated in the absence (blank) or presence of 1 nM DHT (control) or with test compounds (sample) of the present invention in concentrations ranging from 10<sup>-10</sup> to 10<sup>-5</sup> M. Duplicates were used for each sample. The compound dilutions were performed on a Biomek 2000 laboratory work station. Seventy-two h later 0.44 uCi. of [<sup>3</sup>H]-Thymidine (Amersham) was added per well and incubated for another 24 h followed by trypsinization, harvesting of the cells onto GF/B filters. Micro-scint PS were added to the filters before counting them on a Beckman TopCount.

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The % Inhibition was calculated as:

$$\% \text{ Inhibition} = 100 \times (1 - [\text{average}_{\text{control}} - \text{average}_{\text{blank}} / \text{average}_{\text{sample}} - \text{average}_{\text{blank}}])$$

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Data was plotted and the concentration of compound that inhibited 50% of the [<sup>3</sup>H]-Thymidine incorporation was quantified (IC<sub>50</sub>).



### Murine Breast Cell Proliferation Assay

The ability of compounds of the present invention ("test compounds") to modulate the function of the AR was determined by testing said compounds in a proliferation assay using the androgen responsive murine breast cell line derived from the Shionogi tumor, Hiraoka *et al.*, *Cancer Res.*, **47**, 6560-6564 (1987). Stable AR dependent clones of the parental Shionogi line were established by passing tumor fragments under the general procedures originally described in Tetuo, *et. al.*, *Cancer Research* **25**, 1168-1175 (1965). From the above procedure, one stable line, SC114, was isolated, characterized and utilized for the testing of example compounds. SC114 cells were incubated with or without the test compounds for 72 h and the amount of [3H]-thymidine incorporated into DNA was quantified as a surrogate endpoint to assess the number of cells and therefore the proliferation rate as described in Suzuki *et. al.*, *J. Steroid Biochem. Mol. Biol.* **37**, 559-567 (1990). The SC114 cell line was maintained in MEM containing  $10^{-8}$  M testosterone and 2% DCC-treated FCS. For the assay, cells were plated in 96-well microplates in the maintenance media and incubated at 37 °C. On the following day, the medium was changed to serum free medium [Ham's F-12:MEM (1;1, v/v) containing 0.1% BSA] with (antagonist mode) or without (agonist mode)  $10^{-8}$  M testosterone and the test compounds of the present invention in concentrations ranging from  $10^{-10}$  to  $10^{-5}$  M. Duplicates were used for each sample. The compound dilutions were performed on a Biomek 2000 laboratory work station. Seventy two h later 0.44uCi of [3H]-Thymidine (Amersham) was added per well and incubated for another 2 h followed by trypsinization, and harvesting of the cells onto GF/B filters. Micro-scint PS were added to the filters before counting them on a Beckman TopCount.

For the antagonist mode, the % Inhibition was calculated as:

$$\% \text{ Inhibition} = 100 \times (1 - [\text{average}_{\text{sample}} - \text{average}_{\text{blank}} / \text{average}_{\text{control}} - \text{average}_{\text{blank}}])$$

Data was plotted and the concentration of compound that inhibited 50% of the [3H]-Thymidine incorporation was quantified ( $\text{IC}_{50}$ ).

For the agonist mode % Control was referred as the effect of the tested compound compared to the maximal effect observed with the natural hormone, in this case DHT, and was calculated as:

5     
$$\% \text{ Control} = 100 \times (\text{average}_{\text{sample}} - \text{average}_{\text{blank}}) / (\text{average}_{\text{control}} - \text{average}_{\text{blank}})$$

Data was plotted and the concentration of compound that inhibited 50% of the [<sup>3</sup>H]-Thymidine incorporation was quantified (EC<sub>50</sub>).

10     ***In Vitro* Assay to Measure GR-Induced AP-1 Transrepression**

The AP-1 assay is a cell-based luciferase reporter assay. A549 cells, which contain endogenous glucocorticoid receptor, were stably transfected with an AP-1 DNA binding site attached to the luciferase gene. Cells are then grown in RPMI + 10% fetal calf serum (charcoal-treated) + Penicillin/Streptomycin with 0.5mg/mL  
15     geneticin. Cells are plated the day before the assay at approximately 40000 cells/well. On assay day, the media is removed by aspiration and 20 µL assay buffer (RPMI without phenol red + 10% FCS (charcoal-treated) + Pen/Strep) is added to each well. At this point either 20 µL assay buffer (control experiments), the compounds of the present invention ("test compounds") (dissolved in DMSO and added at varying  
20     concentrations) or dexamethasone (100 nM in DMSO, positive control) are added to each well. The plates are then pre-incubated for 15 min at 37 °C, followed by stimulation of the cells with 10 ng/mL PMA. The plates are then incubated for 7 h at 37 °C after which 40 µL luciferase substrate reagent is added to each well. Activity is measured by analysis in a luminometer as compared to control experiments treated  
25     with buffer or dexamethasone. Activity is designated as % inhibition of the reporter system as compared to the buffer control with 10 ng/mL PMA alone. The control, dexamethasone, at a concentration of ≤10 µM typically suppresses activity by 65%. Test compounds which demonstrate an inhibition of PMA induction of 50% or greater at a concentration of test compound of ≤10 µM are deemed active.

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## IN VIVO ASSAYS

## Levator Ani &amp; Wet Prostate Weight Assay AR Agonist Assay

The activity of compounds of the present invention as AR agonists was investigated in an immature male rat model, a recognized test of anabolic effects in muscle and sustaining effects in sex organs for a given compound, as described in L. 5 G. Hershberger et al., *Proc. Soc. Expt. Biol. Med.*, **83**, 175 (1953); B. L. Beyler et al, "Methods for evaluating anabolic and catabolic agents in laboratory animals", *J. Amer. Med. Women's Ass.*, **23**, 708 (1968); H. Fukuda et al., "Investigations of the levator ani muscle as an anabolic steroid assay", *Nago Dai. Yak. Ken. Nem.* **14**, 84 10 (1966) the disclosures of which are herein incorporated by reference.

The basis of this assay lies in the well-defined action of androgenic agents on the maintenance and growth of muscle tissues and sexual accessory organs in animals and man. Androgenic steroids, such as testosterone (T), have been well characterized for their ability to maintain muscle mass. Treatment of animals or humans after 15 castrations with an exogenous source of T results in a reversal of muscular atrophy. The effects of T on muscular atrophy in the rat levator ani muscle have been well characterized. M. Masuoka et al., "Constant cell population in normal, testosterone deprived and testosterone stimulated levator ani muscles" *Am. J. Anat.* **119**, 263 (1966); Z. Gori et al., "Testosterone hypertrophy of levator ani muscle of castrated 20 rats. I. Quantitative data" *Boll. -Soc. Ital. Biol. Sper.* **42**, 1596 (1966); Z. Gori et al., "Testosterone hypertrophy of levator ani muscle of castrated rats. II. Electron-microscopic observations" *Boll. -Soc. Ital. Biol. Sper.* **42**, 1600 (1966); A. Boris et al., *Steroids* **15**, 61 (1970). As described above, the effects of androgens on maintenance of male sexual accessory organs, such as the prostate and seminal 25 vesicles, is well described. Castration results in rapid involution and atrophy of the prostate and seminal vesicles. This effect can be reversed by exogenous addition of androgens. Since both the levator ani muscle and the male sex organs are the tissues most responsive to the effects of androgenic agents, this model is used to determine the androgen dependent reversal of atrophy in the levator ani muscle and the sex 30 accessory organs in immature castrated rats. Sexually mature rats (200-250 g, 6-8 weeks-old, Sprague-Dawley, Harlan) were acquired castrated from the vendor

(Taconic). The rats were divided into groups and treated daily for 7 to 14 days with one of the following:

1. Control vehicle
2. Testosterone Propionate (TP) (3 mg/rat/day, subcutaneous)
- 5 3. TP plus Bicalutamide (administered p.o. in PEGTW, QD), a recognized antiandrogen, as a reference compound.
4. To demonstrate antagonist activity, a compound of the present invention ("test compound") was administered (p.o. in PEGTW, QD) with TP (s.c. as administered in group 2) in a range of doses.
- 10 5. To demonstrate agonist activity a compound of the present invention ("test compound") was administered alone (p.o. in PEGTW, QD) in a range of doses.

At the end of the 7-14-day treatment, the animals were sacrificed by carbon dioxide, and the levator ani, seminal vesicle and ventral prostate weighed. To compare data from different experiments, the levator ani muscle and sexual organ weights were first standardized as mg per 100 g of body weight, and the increase in organ weight induced by TP was considered as the maximum increase (100%).  
15 Super-anova (one factor) was used for statistical analysis.

The gain and loss of sexual organ weight reflect the changes of the cell number (DNA content) and cell mass (protein content), depending upon the serum androgen concentration. See Y. Okuda et al., *J. Urol.*, **145**, 188-191 (1991), the disclosure of which is herein incorporated by reference. Therefore, measurement of organ wet weight is sufficient to indicate the bioactivity of androgens and androgen antagonist. In immature castrated rats, replacement of exogenous androgens increases levator ani, seminal vesicles (SV) and prostate in a dose dependent manner.  
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25 The maximum increase in organ weight was 4 to 5-fold when dosing 3 mg/rat/day of testosterone (T) or 1 mg/rat/day of testosterone propionate (TP) for 3 days. The EC<sub>50</sub> of T and TP were about 1 mg and 0.03 mg, respectively. The increase in the weight of the VP and SV also correlated with the increase in the serum T and DHT concentration. Although administration of T showed 5-times higher serum concentrations of T and DHT at 2 h after subcutaneous injection than that of  
30 TP, thereafter, these high levels declined very rapidly. In contrast, the serum

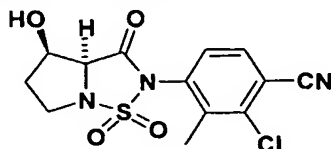
concentrations of T and DHT in TP-treated animals were fairly consistent during the 24 h, and therefore, TP showed about 10-30-fold higher potency than free T.

The following examples serve to better illustrate, but not limit, some of the  
5 preferred embodiments of the invention.

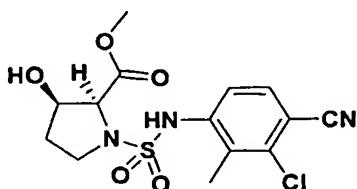
## EXAMPLES

## EXAMPLE 1

5 4-[(3a*S*,4*R*)-4-Hydroxy-1,1,3-trioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile



1A. (2*S*,3*R*)-1-(3-Chloro-4-cyano-2-methyl-phenylsulfamoyl)-3-hydroxy-pyrrolidine-2-carboxylic acid methyl ester

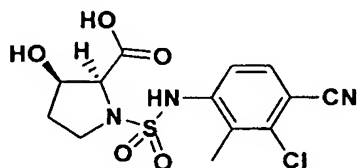


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To a solution of 4-amino-2-chloro-3-methyl-benzonitrile (100 mg, 0.600 mmol) in 3 mL of CH<sub>2</sub>Cl<sub>2</sub> at rt was added chlorosulfonic acid (44 µL, 0.660 mmol) followed by PCl<sub>5</sub> (125 mg, 0.600 mmol) and the suspension refluxed for 30 min. The suspension was cooled to rt and added dropwise to a solution of 0.25 mL of triethylamine in 1 mL of CH<sub>2</sub>Cl<sub>2</sub> cooled to 0 °C. Diisopropylethylamine (0.21 mL, 1.20 mmol) was added followed by *cis*-3-hydroxyproline methyl ester, HCl salt (120 mg, 0.660 mmol) and the solution stirred at 0 °C for 30 min. The reaction mixture was washed with brine, dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 98:2, 95:5 and 90:10) to afford the title compound (97 mg) as a beige solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 1.95-2.02 (m, 1H), 2.07-2.13 (m, 1H), 2.41 (s, 3H), 3.50-3.57 (m, 2H), 3.66 (s, 3H), 4.44 (d, *J* = 7.2, 1H), 4.55-4.59 (m, 1H), 7.63 (d, *J* = 8.8, 1H), 7.70 (d, *J* = 8.8, 1H); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 15.40, 33.92, 47.79, 52.51, 67.04, 73.19, 110.10, 117.32, 122.15, 131.19, 132.88, 137.90, 143.30, 171.80; HPLC a) column: Phenomenex ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90%

H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV detection at 220 nm, 2.57 min retention time; HPLC b) column: YMC S5 ODS Combiscreen C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA, 1 min hold; 4 mL/min, UV detection at 220 nm, 2.50 min retention time (93%); MS (ES) *m/z* 374 [M+H]<sup>+</sup>.

**1B. (2S,3R)-1-(3-Chloro-4-cyano-2-methyl-phenylsulfamoyl)-3-hydroxy-pyrrolidine-2-carboxylic acid methyl ester**

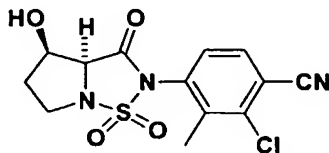


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A solution of 1A (109 mg, 0.292 mmol) in 15 mL of 1.6 N NaOH was stirred at rt for 1 h. The reaction mixture was acidified to pH 2 with 10% HCl and extracted with EtOAc. The organic layer was dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure to afford the title compound (97 mg) as a beige solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 1.96-2.03 (m, 1H), 2.06-2.12 (m, 1H), 2.42 (s, 3H), 3.54-3.56 (m, 2H), 4.42 (d, *J* = 6.6, 1H), 4.57-4.61 (m, 1H), 7.60 (d, *J* = 8.3, 1H), 7.72 (d, *J* = 8.8, 1H); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 15.34, 34.13, 47.88, 67.35, 73.03, 109.87, 117.40, 121.84, 130.90, 132.88, 137.86, 143.52, 172.96; HPLC a) column: Phenomenex LUNA C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV detection at 220 nm, 2.16 min retention time; HPLC b) column: Shimadzu Shim-Pack VP-ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA, 1 min hold; 4 mL/min, UV detection at 220 nm, 2.23 min retention time (100%); MS (ES) *m/z* 360 [M+H]<sup>+</sup>.

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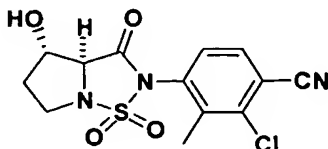
**1C. 4-[(3aS,4R)-4-Hydroxy-1,1,3-trioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile**



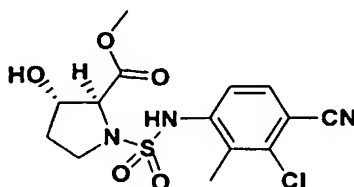
- 5 To a suspension of **1B** (82 mg, 0.228 mmol) in 5 mL of acetonitrile at rt was added DCC (47 mg, 0.228 mmol) followed by *p*-nitrophenol (63 mg, 0.456 mmol). The suspension was refluxed for 1 h, cooled to rt and filtered. The filtrate was concentrated under reduced pressure and the residue dissolved in EtOAc, washed with water and brine, dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure.
- 10 The residue was purified by flash chromatography (silica gel, EtOAc/Hexanes, 50:50 and 75:25) to afford the title compound (61 mg) as an off-white solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 2.21-2.23 (m, 2H), 2.37, 2.39 (s, 3H), 3.61-3.64 (m, 1H), 3.97-4.04 (m, 1H), 4.66, 4.68 (s, 1H), 4.79 (d, *J* = 3.9, 1H), 7.45, 7.55 (d, *J* = 8.3, 1H), 7.83 (d, *J* = 8.3, 1H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 15.47, 15.94, 35.32, 35.44, 48.21, 48.36, 69.77, 69.82, 73.74, 114.68, 115.59, 129.50, 132.70, 132.90, 134.25, 136.92, 138.96, 163.11, 163.59; HPLC a) column: PhenomineX C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV detection at 220 nm, 2.65 min retention time; HPLC b) column: Shimadzu Shim-Pack VP-ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA, 1 min hold; 4 mL/min, UV detection at 220 nm, 2.52 min retention time (100%); HPLC c) column: Daicel Chiralcel OD 4.6 x 250 mm, Isocratic 25% Isopropanol/Hexanes, 30 min, 1 mL/min, UV detection at 220 nm, 11.86 min retention time (99%); MS (ES) *m/z* 340 [M-H]<sup>+</sup>.
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## EXAMPLE 2

4-[(3a*S*,4*S*)-4-Hydroxy-1,1,3-trioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile

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2A. (2*S*,3*S*)-1-(3-Chloro-4-cyano-2-methyl-phenylsulfamoyl)-3-hydroxy-pyrrolidine-2-carboxylic acid methyl ester

10 To a suspension of 4-amino-2-chloro-3-methyl-benzonitrile (500 mg, 3.00 mmol) in 15 mL of CH<sub>2</sub>Cl<sub>2</sub> at rt was added chlorosulfonic acid (0.22 mL, 3.30 mmol) followed by PCl<sub>5</sub> (625 mg, 3.00 mmol) and the suspension refluxed for 1 h. The suspension was cooled to rt and added dropwise to a solution of 1.25 mL of triethylamine in 5 mL of CH<sub>2</sub>Cl<sub>2</sub> cooled to 0 °C. Diisopropylethylamine (1.04 mL,

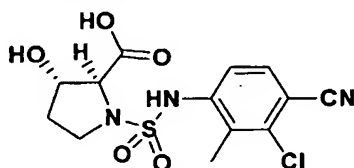
15 6.00 mmol) was added followed by *trans*-3-hydroxyproline methyl ester, HCl salt (600 mg, 3.30 mmol) and the solution stirred at 0 °C for 1.5 h. The reaction mixture was washed with brine, dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure. The residue was purified by prep HPLC (reverse phase silica gel, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA) to afford the title

20 compound (74 mg) as a yellow oil. <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 1.81-1.85 (m, 1H), 1.95-2.02 (m, 1H), 2.32 (s, 3H), 3.43-3.53 (m, 2H), 3.58 (s, 3H), 4.11 (s, 1H), 4.27 (d, *J* = 3.9, 1H), 7.50 (d, *J* = 8.8, 1H), 7.60 (d, *J* = 8.8, 1H); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 15.49, 33.79, 48.21, 52.97, 71.17, 75.64, 110.08, 117.35, 122.35, 131.45, 132.80, 137.87, 143.45, 172.46; HPLC a) column: Phenomenex ODS C18 4.6 x 50 mm, 4 min

25 gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV detection at 220 nm, 2.60 min retention time; HPLC b)

column: Shimadzu Shim-Pack VP-ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA, 1 min hold; 4 mL/min, UV detection at 220 nm, 2.45 min retention time (95%); HPLC c) column: Daicel Chiralcel OD 4.6 x 250 mm, Isocratic 25% Isopropanol/Hexanes, 30 min, 1 mL/min, UV detection at 220 nm, 9.00 min retention time; MS (ES) *m/z* 374 [M+H]<sup>+</sup>.

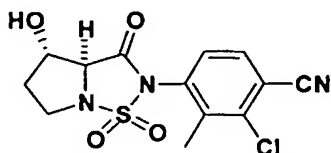
**2B. (2S,3S)-1-(3-Chloro-4-cyano-2-methyl-phenylsulfamoyl)-3-hydroxy-pyrrolidine-2-carboxylic acid methyl ester**



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A solution of the ester (70 mg, 0.187 mmol) in 5 mL of 1.6 N NaOH was stirred at rt for 1 h. The reaction mixture was acidified to pH 2 with 10% HCl and extracted with EtOAc. The organic layer was dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure to afford the title compound (67 mg) as a colorless oil. <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 1.92-1.95 (m, 1H), 2.05-2.12 (m, 1H), 2.41 (s, 3H), 3.54-3.63 (m, 2H), 4.21 (s, 1H), 4.42 (d, *J* = 3.9, 1H), 7.58 (d, *J* = 8.8, 1H), 7.72 (d, *J* = 8.8, 1H); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 15.39, 33.78, 48.13, 71.44, 75.76, 109.77, 117.42, 121.87, 130.98, 132.81, 137.81, 143.66, 173.71; HPLC a) column: Phenominex ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV detection at 220 nm, 2.38 min retention time; HPLC b) column: Shimadzu Shim-Pack VP-ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA, 1 min hold; 4 mL/min, UV detection at 220 nm, 2.23 min retention time (100%); HPLC c) column: Daicel Chiralcel OD 4.6 x 250 mm, Isocratic 25% Isopropanol/Hexanes, 30 min, 1 mL/min, UV detection at 220 nm, 9.00 min retention time; MS (ES) *m/z* 360 [M+H]<sup>+</sup>.

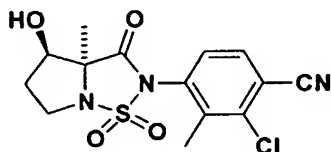
2C. 4-[(3a*S*,4*S*)-4-Hydroxy-1,1,3-trioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile



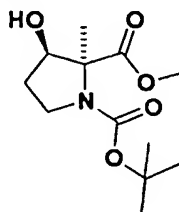
5 To a suspension of the acid (65 mg, 0.181 mmol) in 4 mL of acetonitrile at rt was added DCC (37 mg, 0.181 mmol) followed by *p*-nitrophenol (50 mg, 0.361 mmol). The suspension was refluxed for 1 h, cooled to rt and filtered. The filtrate was concentrated under reduced pressure and the residue dissolved in EtOAc, washed with water and brine, dried (MgSO<sub>4</sub>), filtered and concentrated under reduced  
10 pressure. The residue was purified by flash chromatography (silica gel, EtOAc/hexanes, 25:75 and 75:25) to afford the title compound (49 mg) as a white foam. <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 1.96-2.14 (m, 2H), 2.30, 2.32 (s, 3H), 3.72-3.81 (m, 1H), 4.04-4.11 (m, 1H), 4.63, 4.68 (s, 1H), 4.78, 4.80 (s, 1H), 7.48, 7.52 (d, *J* = 8.3, 1H), 7.81, 7.82 (d, *J* = 8.3, 1H); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 15.96, 16.50, 34.17, 34.24, 52.25,  
15 75.07, 75.15, 75.36, 75.64, 116.37, 116.68, 116.89, 129.59, 130.91, 133.32, 133.48, 135.53, 136.29, 138.77, 140.12, 167.18, 168.00; HPLC a) column: Phenomenex C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV detection at 220 nm, 2.83 min retention time; HPLC b) column: Shimadzu Shim-Pack VP-ODS C18 4.6 x 50 mm, 4 min  
20 gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA, 1 min hold; 4 mL/min, UV detection at 220 nm, 2.76 min retention time (99%); HPLC c) column: Daicel Chiralcel OD 4.6 x 250 mm, Isocratic 25% Isopropanol/Hexanes, 30 min, 1 mL/min, UV detection at 220 nm, 10.87 min retention time (99%); MS (ES) *m/z* 340 [M-H]<sup>+</sup>.

25

## EXAMPLE 3

4-[(3*aS*,4*R*)-4-Hydroxy-3*a*-methyl-1,1,3-trioxohexahydrothia-2,6*a*-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile

5

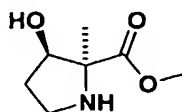
3A. (2*S*,3*R*)-*N*-*tert*-butoxycarbonyl-3-hydroxy-2-methyl-pyrrolidine-2-carboxylic acid methyl ester

10 To a solution of 2 M LDA in THF (1.2 mL, 2.45 mmol) cooled to  $-50^{\circ}\text{C}$  was added a solution of the proline (200 mg, 0.815 mmol) in 1 mL of THF dropwise. The solution was stirred at  $-10^{\circ}\text{C}$  for 25 min and then at  $0^{\circ}\text{C}$  for 5 min. The solution was cooled to  $-30^{\circ}\text{C}$  and a solution of Iodomethane (76  $\mu\text{L}$ , 1.22 mmol) in HMPA (0.20 mL, 1.14 mmol) was added dropwise. The solution was stirred at  $0^{\circ}\text{C}$  for 1 h

15 and then at rt overnight. The reaction mixture was diluted with saturated  $\text{NH}_4\text{Cl}$  solution and extracted with EtOAc. The organic layer was washed with brine, dried ( $\text{MgSO}_4$ ), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel,  $\text{CH}_2\text{Cl}_2/\text{CH}_3\text{OH}$ , 98:2, 95:5 and 90:10) to afford the title compound (131 mg) as an orange oil.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  1.39, 1.43 (s, 9H), 1.57-1.58 (s, 3H), 1.90-1.98 (m, 1H), 2.02-2.08 (m, 1H), 3.30-3.39 (m, 1H), 3.63-3.79 (m, 4H), 4.07-4.13 (m, 1H);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  21.71, 22.82, 28.59, 28.73, 31.25, 31.73, 45.48, 46.05, 52.43, 52.48, 70.47, 70.54, 80.95, 81.04, 81.57, 81.72, 155.71, 155.77, 173.85, 173.93; HPLC a) column: Phenominex C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90%  $\text{H}_2\text{O}$ /0.1% TFA to 90% MeOH/10%  $\text{H}_2\text{O}$ /0.1% TFA; 1 min hold, 4 mL/min UV detection at 220 nm, 2.42 min retention time; MS (ES)  $m/z$  260  $[\text{M}+\text{H}]^+$ .

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**3B. (2S,3R) -3-hydroxy-2-methyl-pyrrolidine-2-carboxylic acid methyl ester, trifluoroacetic acid salt**

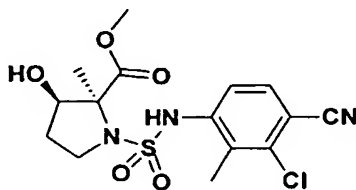


5

To a solution of the proline (130 mg, 0.501 mmol) in 1.5 mL of CH<sub>2</sub>Cl<sub>2</sub> cooled to 0 °C was added 1 mL of TFA dropwise. The solution was stirred at 0 °C for 2 h and concentrated under reduced pressure. The residue was azeotroped with CH<sub>2</sub>Cl<sub>2</sub> (3x3 mL) and CH<sub>2</sub>Cl<sub>2</sub>/toluene (1:1; 3x6 mL) to afford the TFA salt of the title compound (158 mg) as an orange oil. <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 1.53 (s, 3H), 2.06-2.11 (m, 1H), 2.40-2.47 (m, 1H), 3.44-3.49 (m, 1H), 3.55-3.61 (m, 1H), 3.85 (s, 3H), 4.36 (d, *J* = 3.9, 1H); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 18.96, 32.45, 43.60, 53.83, 75.38, 77.23, 170.61; HPLC a) column: Phenominex C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV detection at 220 nm, 0.20 min retention time; MS (ES) *m/z* 160 [M+H]<sup>+</sup>.

15

**3C. (2S,3R)-1-(3-Chloro-4-cyano-2-methyl-phenylsulfamoyl)-3-hydroxy-2-methyl-pyrrolidine-2-carboxylic acid methyl ester**



20

To a suspension of 4-amino-2-chloro-3-methyl-benzonitrile (75 mg, 0.450 mmol) in 2.25 mL of CH<sub>2</sub>Cl<sub>2</sub> at rt was added chlorosulfonic acid (33 uL, 0.495 mmol) followed by PCl<sub>5</sub> (94 mg, 0.450 mmol) and the suspension refluxed for 30 min. The suspension was cooled to rt and added dropwise to a solution of 0.19 mL of triethylamine in 1 mL of CH<sub>2</sub>Cl<sub>2</sub> cooled to 0 °C. A solution of diisopropylethylamine (0.16 mL, 0.900 mmol) and **3B** (137 mg, 0.501 mmol) in 1 mL of CH<sub>2</sub>Cl<sub>2</sub> was added and the solution stirred at 0 °C for 30 min. The reaction mixture was washed with

25

water and brine, dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure.

The residue was purified by flash chromatography (silica gel, CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 98:2) to afford the title compound (120 mg) as an orange foam. <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 1.52

(s, 3H), 1.97-2.17 (m, 2H), 2.44 (s, 3H), 3.44-3.49 (m, 1H), 3.67-3.80 (m, 5H), 7.63

5 (s, 2H); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 15.50, 23.03, 31.71, 47.91, 52.86, 74.10, 81.37, 110.31, 117.29, 122.98, 131.91, 132.81, 137.98, 143.44, 171.94; HPLC a) column:

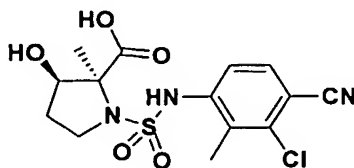
Phenominex C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV detection at 220 nm,

2.62 min retention time; HPLC b) column: Shimadzu Shim-Pack VP-ODS C18 4.6 x

10 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10%

H<sub>2</sub>O/0.1% TFA, 1 min hold; 4 mL/min, UV detection at 220 nm, 2.55 min retention time (85%); MS (ES) *m/z* 388 [M+H]<sup>+</sup>.

3D. (2S,3R)-1-(3-Chloro-4-cyano-2-methyl-phenylsulfamoyl)-3-hydroxy-2-methyl-pyrrolidine-2-carboxylic acid



A solution of 3C (120 mg, 0.309 mmol) in 8 mL of 1.6 N NaOH was stirred at rt overnight. The reaction mixture was acidified to pH 2 with 10% HCl and extracted

20 with EtOAc. The organic layer was dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure to afford the title compound (76 mg) as an orange oil. <sup>1</sup>H NMR

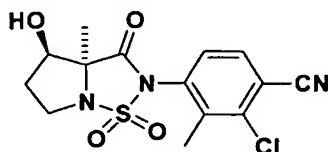
(CD<sub>3</sub>OD) δ 1.58 (s, 3H), 1.98-2.04 (m, 1H), 2.13-2.19 (m, 1H), 2.43 (s, 3H), 3.49-3.54 (m, 1H), 3.65-3.70 (m, 1H), 4.12 (t, *J* = 6.1, 1H), 7.59 (d, *J* = 8.3, 1H), 7.65 (d, *J* = 8.8, 1H); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 15.33, 23.61, 31.78, 48.13, 74.81, 81.10, 109.72,

25 117.40, 122.04, 130.98, 132.84, 137.85, 143.90, 175.19; HPLC a) column:

Phenominex ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV detection at 220 nm, 2.55 min retention time; HPLC b) column: Shimadzu Shim-Pack VP-ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10%

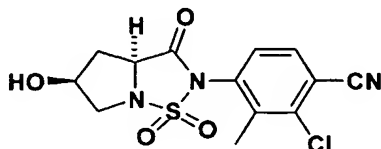
H<sub>2</sub>O/0.1% TFA, 1 min hold; 4 mL/min, UV detection at 220 nm, 2.40 min retention time (89%); MS (ES) *m/z* 374 [M+H]<sup>+</sup>.

3E. 4-[(3*aS*,4*R*)-4-Hydroxy-3*a*-methyl-1,1,3-trioxohexahydrothia-2,6*a*-  
5 diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile

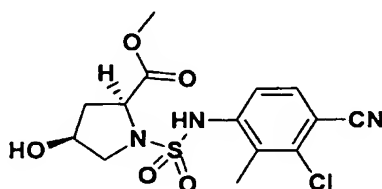


- To a suspension of **3D** (70 mg, 0.187 mmol) in 4 mL of acetonitrile at rt was added DCC (39 mg, 0.187 mmol) followed by *p*-nitrophenol (53 mg, 0.374 mmol).
- 10 The suspension was refluxed for 1 h, cooled to rt and filtered. The filtrate was concentrated under reduced pressure and the residue dissolved in EtOAc, washed with water and brine, dried (MgSO<sub>4</sub>), filtered and the filtrate concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, EtOAc/Hexanes, 50:50) to afford the title compound (48 mg) as a yellow foam. <sup>1</sup>H
- 15 NMR (CD<sub>3</sub>OD) δ 1.59, 1.64 (s, 3H), 2.14-2.18 (m, 1H), 2.33-2.39 (m, 4H), 3.62-3.68 (m, 1H), 3.98-4.07 (m, 1H), 4.29, 4.31 (d, *J* = 3.3, 1H), 7.45, 7.52 (d, *J* = 8.3, 1H), 7.81, 7.82 (d, *J* = 8.3, 1H); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 16.11, 16.25, 22.94, 23.24, 34.60, 50.21, 50.67, 77.10, 77.26, 82.46, 82.66, 116.44, 116.55, 129.95, 131.05, 133.22, 133.35, 136.32, 138.82, 140.34, 141.02, 169.27; HPLC a) column: Phenominex
- 20 LUNA C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV detection at 220 nm, 2.90 min retention time; HPLC b) column: Shimadzu Shim-Pack VP-ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA, 1 min hold; 4 mL/min, UV detection at 220 nm, 2.75 min retention time (98%);
- 25 HPLC c) column: Daicel Chiralcel OD 4.6 x 250 mm, Isocratic 25% Isopropanol/Hexanes, 30 min, 1 mL/min, UV detection at 220 nm, 6.96 min retention time (98%); MS (ES) *m/z* 356 [M+H]<sup>+</sup>.

## EXAMPLE 4

4-[(3*aS*,5*S*)-5-Hydroxy-1,1,3-trioxohexahydrothia-2,6*a*-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile

5

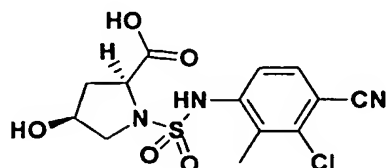
4A. (2*S*,4*S*)-1-(3-Chloro-4-cyano-2-methyl-phenylsulfamoyl)-4-hydroxy-pyrrolidine-2-carboxylic acid methyl ester

- 10 To a solution of 4-amino-2-chloro-3-methyl-benzonitrile (150 mg, 0.900 mmol) in 4.5 mL of CH<sub>2</sub>Cl<sub>2</sub> at rt was added chlorosulfonic acid (66  $\mu$ L, 0.990 mmol) followed by PCl<sub>5</sub> (187 mg, 0.900 mmol) and the suspension refluxed for 1.5 h. The suspension was cooled to rt and added dropwise to a solution of 0.31 mL of triethylamine in 2 mL of CH<sub>2</sub>Cl<sub>2</sub> cooled to 0 °C. A solution of diisopropylethylamine
- 15 (0.37 mL, 1.80 mmol) and *cis*-4-hydroxyproline methyl ester, HCl salt (180 mg, 0.990 mmol) in 2 mL of CH<sub>2</sub>Cl<sub>2</sub> was added dropwise and the solution stirred at 0 °C for 30 min. The reaction mixture was washed with water and brine, dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, CH<sub>2</sub>Cl<sub>2</sub>/CH<sub>3</sub>OH, 98:2) to afford the title compound (61
- 20 mg) as an orange foam. <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  2.34-2.43 (m, 5H) 3.59-3.62 (m, 1H), 3.65-3.69 (m, 4H), 4.38-4.41 (m, 1H), 4.52-4.55 (m, 1H), 7.62 (d, *J* = 8.3, 1H), 7.72 (d, *J* = 8.8, 1H); <sup>13</sup>C NMR (CD<sub>3</sub>OD)  $\delta$  15.34, 39.87, 52.97, 57.29, 61.84, 70.97, 109.87, 117.36, 121.79, 130.83, 132.92, 137.87, 143.55, 174.54; HPLC a) column: Phenomenex ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV detection at 220
- 25 nm, 2.60 min retention time; HPLC b) column: Shimadzu Shim-Pack VP-ODS C18



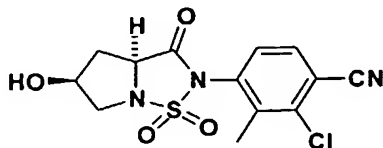
4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA, 1 min hold; 4 mL/min, UV detection at 220 nm, 2.45 min retention time (70%); MS (ES) *m/z* 374 [M+H]<sup>+</sup>.

5    **4B.    (2S,4S)-1-(3-Chloro-4-cyano-2-methyl-phenylsulfamoyl)-4-hydroxy-pyrrolidine-2-carboxylic acid**



A solution of 4A (60 mg, 0.160 mmol) in 4 mL of 1.6 N NaOH was stirred at  
 10    rt for 1 h. The reaction mixture was acidified to pH 2 with 10% HCl. A solid  
 precipitated which was filtered, washed with water and dried to afford the title  
 compound (58 mg) as an orange solid. <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 2.38-2.42 (m, 5H) 3.31-  
 3.34 (m, 1H), 3.62-3.65 (m, 1H), 4.40-4.42 (m, 1H), 4.49-4.52 (m, 1H), 7.60 (d, *J* =  
 8.8, 1H), 7.71 (d, *J* = 8.8, 1H); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 15.28, 39.89, 57.29, 61.95,  
 15    71.03, 109.61, 117.42, 121.42, 130.55, 132.93, 137.84, 143.77, 175.99; HPLC a)  
 column: Phenomenex ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90%  
 H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV  
 detection at 220 nm, 2.40 min retention time; HPLC b) column: Shimadzu Shim-Pack  
 VP-ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90%  
 20    MeOH/10% H<sub>2</sub>O/0.1% TFA, 1 min hold; 4 mL/min, UV detection at 220 nm, 2.23  
 min retention time (63%); MS (ES) *m/z* 360 [M+H]<sup>+</sup>.

**4C.    4-[(3a*S*,5*S*)-5-Hydroxy-1,1,3-trioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile**



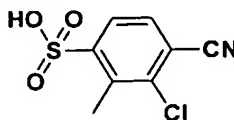
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To a solution of **4B** (57 mg, 0.158 mmol) in 5 mL of acetonitrile at rt was added DCC (33 mg, 0.158 mmol) followed by *p*-nitrophenol (44 mg, 0.317 mmol). The suspension was refluxed for 1 h, cooled to rt and filtered. The filtrate was concentrated under reduced pressure and the residue dissolved in EtOAc, washed with water and brine, dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, EtOAc/Hexanes, 50:50 and 75:25) to afford the title compound (28 mg) as a beige solid. <sup>1</sup>H NMR (DMSO-d<sub>6</sub>) δ 2.25, 2.28 (s, 3H), 2.31- 2.38 (m, 2H), 3.46-3.50 (m, 1H), 3.86 (d, *J* = 12.1, 1H), 4.24 (s, 1H), 4.93, 5.07 (d, *J* = 7.2, 1H), 5.42, 5.51 (s, 1H), 7.27, 7.50 (d, *J* = 8.2, 1H), 7.98, 8.03 (d, *J* = 8.3, 1H); <sup>13</sup>C NMR (DMSO-d<sub>6</sub>) δ 15.06, 15.74, 37.57, 60.55, 60.72, 64.84, 65.37, 68.30, 114.37, 115.61, 128.85, 129.59, 132.65, 132.79, 134.96, 136.96, 138.35, 139.36, 169.16; HPLC a) column: Phenominex LUNA C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV detection at 220 nm, 2.62 min retention time; HPLC b) column: Shimadzu Shim-Pack VP-ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA, 1 min hold; 4 mL/min, UV detection at 254 nm, 2.45 min retention time (100%); HPLC c) column: Daicel Chiralcel OD 4.6 x 250 mm, Isocratic 25% Isopropanol/Hexanes, 30 min, 1 mL/min, UV detection at 220 nm, 12.65 min retention time (99%); MS (ES) *m/z* 340 [M-H]<sup>-</sup>.

### EXAMPLE 5

#### (2S,3R)-1-(3-Chloro-2-methyl-benzenesulfonyl)-3-hydroxy-pyrrolidine-2-carboxylic acid methyl ester

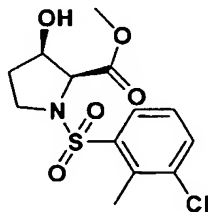
##### 5A. 3-Chloro-2-methyl-benzenesulfonic acid



To a suspension of 4-amino-2-chloro-3-methyl-benzonitrile (500 mg, 3.00 mmol) in 1.8 mL of 6 N HCl at rt was added 2 mL of water followed by a solution of

NaNO<sub>2</sub> (220 mg, 3.13 mmol) in 1 mL of water dropwise and the suspension stirred at rt for 20 min. The suspension was added to a solution of SO<sub>2</sub> in acetic acid and copper(II) chloride dihydrate (60 mg, 3.52 mmol) in 0.15 mL of water. The suspension was stirred at rt for 1.25 h and extracted with EtOAc. The organic layer  
5 was washed with water and brine, dried (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, CH<sub>2</sub>Cl<sub>2</sub>/Hexanes, 50:50, 75:25 and 100:0) to afford the title compound (305 mg) as a white solid. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.84 (s, 3H), 7.70 (d, *J* = 8.8, 1H), 8.07 (d, *J* = 8.8, 1H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 17.51, 114.57, 120.07, 127.11, 131.39, 138.13, 140.19,  
10 147.23; HPLC a) column: Phenomenex LUNA C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV detection at 220 nm, 2.85, 3.37 min retention time; HPLC b) column: Shimadzu Shim-Pack VP-ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA, 1 min hold; 4 mL/min, UV  
15 detection at 220 nm, 2.68, 3.18 min retention time (86%); MS (ES) *m/z* 230 [M-H]<sup>+</sup>.

**5B. (2S,3R)-1-(3-Chloro-2-methyl-benzenesulfonyl)-3-hydroxy-pyrrolidine-2-carboxylic acid methyl ester**



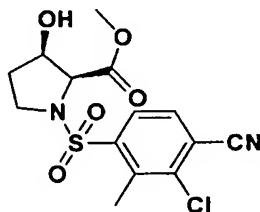
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To a solution of 3-chloro-2-methylbenzenesulfonyl chloride (113 mg, 0.500 mmol) in 1 mL of pyridine at rt was added *cis*-3-hydroxyproline methyl ester, HCl salt (100 mg, 0.550 mmol). The solution was stirred at rt for 30 min, diluted with water and extracted with EtOAc. The organic layer was washed with water and brine, dried  
25 (MgSO<sub>4</sub>), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (silica gel, EtOAc/Hexanes, 50:50) to afford the title compound (104 mg) as a colorless oil. <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 2.00-2.08 (m, 1H), 2.10-2.17 (m, 1H), 2.47 (br s, 1H), 2.64, (s, 3H), 3.51-3.65 (m, 5H), 4.49 (d, *J* = 7.0, 1H),

4.61- 4.66 (m, 1H), 7.19 (t,  $J = 8.4$ , 1H), 7.51 (d,  $J = 7.9$ , 1H), 7.87 (d,  $J = 7.9$ , 1H);  
 $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  16.93, 33.19, 46.29, 52.20, 64.11, 72.59, 126.41, 128.28, 133.83,  
136.41, 137.01, 139.52, 169.65; HPLC a) column: Phenominex ODS C18 4.6 x 50  
mm, 4 min gradient, 10% MeOH/90%  $\text{H}_2\text{O}$ /0.1% TFA to 90% MeOH/10%  $\text{H}_2\text{O}$ /0.1%  
5 TFA; 1 min hold, 4 mL/min UV detection at 220 nm, 2.77 min retention time; HPLC  
b) column: Shimadzu Shim-Pack VP-ODS C18 4.6 x 50 mm, 4 min gradient, 10%  
MeOH/90%  $\text{H}_2\text{O}$ /0.1% TFA to 90% MeOH/10%  $\text{H}_2\text{O}$ /0.1% TFA, 1 min hold; 4  
mL/min, UV detection at 220 nm, 2.68 min retention time (99%); HPLC c) column:  
Daicel Chiralcel OD 4.6 x 250 mm, Isocratic 25% Isopropanol/Hexanes, 30 min, 1  
10 mL/min, UV detection at 220 nm, 5.80 min retention time (99%); MS (ES)  $m/z$  334  
[ $\text{M}+\text{H}$ ] $^+$ .

### EXAMPLE 6

(2S,3R)-1-(3-Chloro-4-cyano-2-methyl-benzenesulfonyl)-3-hydroxy-pyrrolidine-  
15 2-carboxylic acid methyl ester

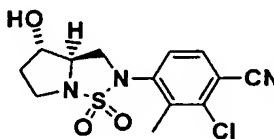


To a solution of 3-Chloro-4-cyano-2-methyl-benzenesulfonic acid (50 mg,  
0.216 mmol) in 1 mL of thionyl chloride cooled to 0 °C was added two drops of  
20 DMF. The solution was stirred for 30 min, concentrated under reduced pressure and  
the residue azeotroped with toluene (3x2 mL). The residue was dissolved in 1 mL of  
pyridine and *cis*-3-hydroxyproline methyl ester, HCl salt (78 mg, 0.429 mmol) was  
added. The solution was stirred at rt overnight and concentrated under reduced  
pressure. The residue was purified by prep HPLC (reverse phase silica gel, 10%  
25 MeOH/90%  $\text{H}_2\text{O}$ /0.1% TFA to 90% MeOH/10%  $\text{H}_2\text{O}$ /0.1% TFA) to afford the title  
compound (16 mg) as a white solid.  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  2.01-2.07 (m, 1H), 2.12-  
2.19 (m, 1H), 2.74, (s, 3H), 3.58, (s, 3H), 3.66 (t,  $J = 7.1$ , 2H), 4.50 (d,  $J = 7.2$ , 1H),  
4.64- 4.67 (m, 1H), 7.81 (d,  $J = 8.3$ , 1H), 8.05 (d,  $J = 8.2$ , 1H);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$

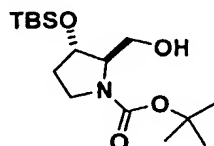
17.75, 33.99, 47.82, 52.43, 66.31, 73.32, 116.27, 118.52, 129.11, 132.58, 138.86, 139.56, 145.66, 171.05; HPLC a) column: Phenominex LUNA C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA; 1 min hold, 4 mL/min UV detection at 220 nm, 2.82 min retention time; HPLC b) column: Shimadzu Shim-Pack VP-ODS C18 4.6 x 50 mm, 4 min gradient, 10% MeOH/90% H<sub>2</sub>O/0.1% TFA to 90% MeOH/10% H<sub>2</sub>O/0.1% TFA, 1 min hold; 4 mL/min, UV detection at 220 nm, 2.68 min retention time (100%); HPLC c) column: Daicel Chiralcel OD 4.6 x 250 mm, Isocratic 25% Isopropanol/Hexanes, 30 min, 1 mL/min, UV detection at 220 nm, 11.29 min retention time (98%); MS (ES) *m/z* 359 [M+H]<sup>+</sup>.

#### EXAMPLE 7

##### 4-[(3*aR*,4*S*)-4-Hydroxy-1,1-dioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile



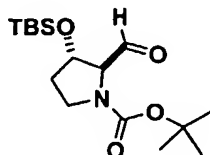
##### 7A. (2*R*,3*S*)-*N*-*tert*-Butyloxycarbonyl-3-(*tert*-butyldimethylsilyloxy)-2-hydroxymethylpyrrolidine



To a solution of (2*S*,3*S*)-*N*-*tert*-Butyloxycarbonyl-3-hydroxy-2-pyrrolidine-carboxylic acid methyl ester (4.3 g, 12 mmol) in THF (75 mL) at -78 °C under nitrogen was added dropwise 1.0 M lithium triethylborohydride in tetrahydrofuran (60 mL, 60 mmol). The reaction was allowed to warm to rt and was stirred for 4 h. The reaction was quenched by pouring over ice (150 g) and stirring for 30 min. The product was extracted into EtOAc and washed with saturated aqueous NaHCO<sub>3</sub>. Organics were dried over MgSO<sub>4</sub>, filtered and concentrated. Purification by flash

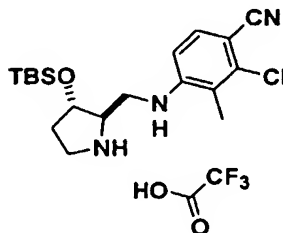
chromatography (silica gel, 0-15% EtOAc in hexanes) provided the title compound (3.3 g).

**7B. (2S,3S)-N-tert-Butyloxycarbonyl-3-(tert-butyldimethylsilanyloxy)-2-formyl-pyrrolidine**



To a solution of **7A** (3.3 g, 9.97 mmol) in 10% CH<sub>3</sub>CN in CH<sub>2</sub>Cl<sub>2</sub> (50 mL) at rt was added NMO (3.0g, 25.6 mmol) followed by tetrapropyl ammonium perruthenate (300 mg, 0.85 mmol). After a mild exotherm the reaction was stirred at  
10 rt for 4 h. The reaction was diluted with hexane (50 mL), mixed well and filtered through a plug of silica gel (~50 g). The silica plug was washed liberally with 30% EtOAc in hexane (500 mL). Solvents were removed under vacuum. Purification by flash chromatography (silica gel, 0-15% EtOAc in hexanes) provided the title  
15 compound (2.94 g).

**7C. (2*R*,3*S*)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(3-chloro-4-cyano-2-methylphenylamino)methyl]pyrrolidine, trifluoroacetic acid salt**

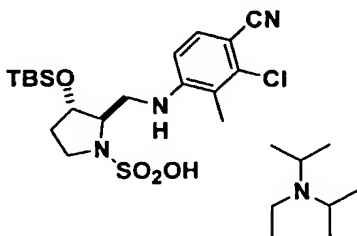


20

To a solution of **7B** (730 mg, 2.21 mmol) in 10% DMF in CH<sub>2</sub>Cl<sub>2</sub> (5.5 mL) at rt was added 4-amino-2-chloro-3-methyl-benzonitrile (400 mg, 2.4 mmol) followed acetic acid (200  $\mu$ L) and sodium triacetoxyborohydride (730 mg, 3.5 mmol). The reaction was stirred for 24 h at rt. The reaction was then extracted with EtOAc and washed with saturated aqueous NaHCO<sub>3</sub>. The organic extracts were dried over MgSO<sub>4</sub> filtered and concentrated. Purification by flash chromatography (silica gel, 0-

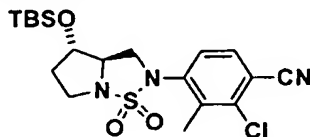
20% EtOAc in hexanes) provided impure (2*R*,3*S*)-*N*-*tert*-Butyloxycarbonyl-3-(*tert*-butyldimethylsilanyloxy)-2-[(3-chloro-4-cyano-2-methyl-phenylamino)-methyl]pyrrolidine as a light brown residue. This residue was taken up in 10% TFA in CH<sub>2</sub>Cl<sub>2</sub> (20 mL) and stirred for 2.5 h at rt. Toluene (10 mL) was added and solvents were removed under vacuum. Purification by preparative HPLC (separated into 4 injections, YMC C18 5  $\mu$ m 30x100mm, 0-100%B, 10 min grad., 2 min. hold, 25 mL/min: A = 10%MeOH/Water + 0.1%TFA, B = 90% MeOH/Water + 0.1%TFA) provided the title compound (267 mg). MS (ES) *m/z* 380.23 [M+1]<sup>+</sup>

10 **7D. (2*R*,3*S*)-3-(*tert*-Butyldimethylsilanyloxy)-2-[(3-chloro-4-cyano-2-methyl-phenylamino)methyl]pyrrolidine-1-sulfonic acid, *N,N,N*-diisopropylethylamine salt**



15 To a solution of 7C (267 mg, 0.53 mmol) in 10% *i*-Pr<sub>2</sub>NEt in CH<sub>2</sub>Cl<sub>2</sub> (5.5 mL) at rt was added 0.75 M chlorosulfonic acid in CH<sub>2</sub>Cl<sub>2</sub> (1.0 mL, 0.75 mmol). The reaction mixture was stirred at rt for 30 minutes. Solvents were removed. Purification by flash chromatography (silica gel, 0-30% EtOAc in hexane flush followed by 5% MeOH in CH<sub>2</sub>Cl<sub>2</sub> to the title compound) provided the title compound 20 (260 mg) as a clear residue. MS (ES) *m/z* 460.20 [M+1]<sup>+</sup>

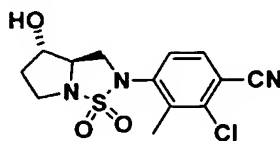
**7E. 4-[(3*aR*,4*S*)-4-(*tert*-Butyldimethylsilanyloxy)-1,1-dioxohexahydrothia-2,6*a*-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile**



To a solution of **7D** (260 mg, 0.44 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and pyridine (3 mL) at rt was added POCl<sub>3</sub> (93 µL, 0.99 mmol). The reaction mixture was stirred at rt for 10 min. The reaction was diluted with CH<sub>2</sub>Cl<sub>2</sub> (5 mL) and *i*-Pr<sub>2</sub>NEt (3mL) was added and the reaction was stirred overnight. Solvents were removed under vacuum.

- 5 Purification by preparative HPLC (separated into 2 injections, YMC C18 5 µm 30x100 mm, 0-100%B, 10 min grad., 2 min. hold, 25 mL/min: A = 10%MeOH/Water + 0.1%TFA, B = 90% MeOH/Water + 0.1%TFA) provided title compound (8 mg) as a clear residue. MS (ES) *m/z* 442.19 [M+1]<sup>+</sup>

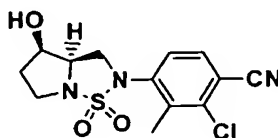
- 10 **7F. 4-[(3a*R*,4*S*)-4-Hydroxy-1,1-dioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile**



- To a solution of **7E** (8 mg, 0.018 mmol) in THF (2 mL) and HOAc (0.5 mL) was treated with 1.0 M TBAF in THF (1 mL, 1.0 mmol). The reaction mixture was stirred at rt for 18 h. The reaction was diluted EtOAc (30 mL) and washed with water (2 x 30 mL). The organics were dried over MgSO<sub>4</sub>, filtered and concentrated. Purification by preparative HPLC (separated into 2 injections, YMC C18 5 µm 20x100mm, 0-100%B, 10 min grad., 2 min. hold, 20 mL/min: A = 10%MeOH/Water + 0.1%TFA, B = 90% MeOH/Water + 0.1%TFA) provided title compound (2.25 mg) as a white solid. MS (ES) *m/z* 328.15 [M+1]<sup>+</sup>
- 15
- 20

#### EXAMPLE 8

- 2-Chloro-4-[(3a*R*,4*R*)-4-hydroxy-1,1-dioxohexahydrothia-2,6a-diazapentalen-2-yl]-3-methylbenzonitrile**
- 25



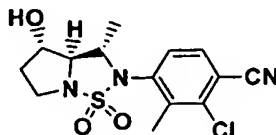


The title compound was prepared from (2*S*,3*R*)-*N*-*tert*-Butyloxycarbonyl-3-hydroxy-2-pyrrolidinecarboxylic acid methyl ester by procedures analogous to those described in Example 7 (7A to 7F). HRMS (ES)  $m/z$  328.0528  $[M+1]^+$ .

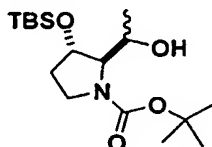
5

**EXAMPLE 9**

**4-[(3*aR*, 3*S*,4*S*)-4-Hydroxy-3-methyl-1,1-dioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile**



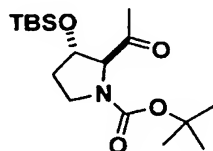
10 **9A. (2*R*,3*S*)-*N*-*tert*-Butyloxycarbonyl-3-(*tert*-butyldimethylsilanyloxy)-2-(1-hydroxyethyl) pyrrolidine**



To a solution of intermediate **7B** (~0.05 M in THF) at -78°C was added dropwise MeMgBr (3.0M in Et<sub>2</sub>O, 3 eq). The reaction was stirred at -78 °C for 1.5-2 h (quench small aliquot in 10%MeOH/DCM, spot TLC -silica gel, 30% EtOAc/Hex – stain with 5% HCl/MeOH, heat and then ninhydrin, heat). Quench reaction by addition of HOAc (3 eq) at -78 °C. Warm to RT, dilute with EtOAc and wash with a 1:1 mixture of sat. aq. NaHCO<sub>3</sub> and sat aq. NaCl. Back extract aqueous layer.

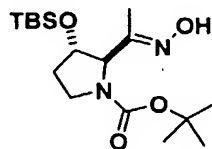
20 Combine organic extracts, dry over MgSO<sub>4</sub>, filter and concentrate. Purification by flash chromatography (silica gel, step gradient 0-10-20% ethylacetate in hexane) provided the title compound.

**9B. (2S,3S)-N-tert-Butyloxycarbonyl-3-(tert-butyldimethylsilyloxy)-2-(1-hydroxyethyl) pyrrolidine**



5 To intermediate **9A** (0.1M solution in 10%CH<sub>3</sub>CN in DCM) add NMO (2 eq) followed by TPAP (0.05 eq). Provide sufficient ventilation and take care to watch for exotherm. Stir reaction at rt for 3-5 h. Dilute by half with hexane and allow TPAP to precipitate. Flash filter through silica gel plug (20 fold mass of starting alcohol) and elute with 10% EtOAc/Hex until product is not observed in eluent. Removal of  
10 solvents gave the title compound. MS (ES) *m/z* 344.31 [M+H]<sup>+</sup>

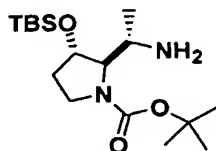
**9C. (2R,3S)-N-tert-Butyloxycarbonyl-3-(tert-butyldimethylsilyloxy)-2-(1-hydroxyimino-ethyl) pyrrolidine**



15

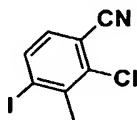
To a solution of **9B** (3.86g, 11.3 mmol) in methanol:water (2:1, 50 mL) was added hydroxylamine hydrochloride salt (3.0 g) followed by pyridine (5.0 mL). The reaction was stirred at rt for 18 hr. Extract product into EtOAc, washing once with sat. aqueous NaHCO<sub>3</sub> and once with water. Back extract the aqueous washings with  
20 EtOAc and combine this with the initial extract. Dry organic extract over MgSO<sub>4</sub>, filter and concentrate. Dilute residue in toluene and remove solvent under vacuum to provide the title compound as a white solid (2.9 g). MS (ES) *m/z* 359.30 [M+H]<sup>+</sup>

**9D. (2R,3S)-N-tert-Butyloxycarbonyl-3-(tert-butyldimethylsilyloxy)-2-((1S)-1-aminoethyl) pyrrolidine**



5 To a solution of 9C (3.6 g, 10.0 mmol) in methanol in a pressure vessel was added Raney Ni in water (~0.2 g), 10% Pd/C (Degussa type, 200 mg), water (7.5 mL) and ammonia in methanol (2.0M, 5 mL). The reaction vessel was carefully evacuated under vacuum until the solvent bubbled gently. Hydrogen gas was then introduced to a pressure of 70 psi and the reaction was stirred for 2 minutes. ). The reaction vessel  
10 was again carefully evacuated under vacuum until the solvent bubbled gently. Hydrogen gas was then introduced to a pressure of 70 psi and the reaction was stirred for 18 h at rt. The reaction was filtered through a pad of celite, taking care to keep the captured catalyst wet with methanol at all times. The celite pad was washed with methanol until the product could no longer be detected in the eluent (TLC, 5% MeOH  
15 in CH<sub>2</sub>Cl<sub>2</sub>, stain with ninhydrin and heat). Solvent was removed under vacuum and purification by flash chromatography (110g ISCO silica gel cartridge, step gradient 0%-5%-10% MeOH in CH<sub>2</sub>Cl<sub>2</sub>) gave the title compound (2.2g): MS (ES) *m/z* 345.35 [M+H]<sup>+</sup>

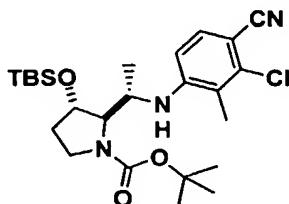
**20 9E. 2-Chloro-4-iodo-3-methyl-benzonitrile**



To a suspension of CuI (7.5 g, 39.3 mmol) in acetonitrile (150 ml) under N<sub>2</sub> at rt was added tert-butylnitrite (5.7 mL, 47.9 mmol). The reaction mixture was heated  
25 to 65 °C for 1 h and then 4-amino-2-chloro-3-methyl-benzonitrile (6.0 g, 36.0 mmol) was added and the reaction was heated at 65 °C for 3 h. The reaction was cooled to rt and filtered through a pad of celite. The celite pad was washed with ethylacetate. The organics were washed twice with water, dried over MgSO<sub>4</sub>, filtered and

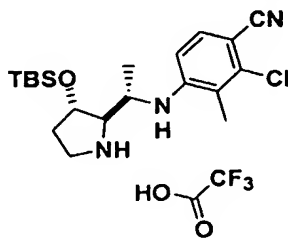
concentrated. Purification by flash chromatography (silica gel, 110 g ISCO, 0-5% ethylacetate in hexane, step gradient) gave the title compound (4.3 g): MS (ES)  $m/z$  278  $[M+H]^+$

5 **9F. (2R,3S)-1-tert- Butyloxycarbonyl-3-(tert-Butyldimethylsilanyloxy)-2-[(1S)-1-(3-chloro-4-cyano-2-methyl-phenylamino)-ethyl]pyrrolidine**



To a solution of **9D** (325 mg, 0.94 mmol) in nitrogen degassed toluene:DMSO  
 10 (2:1, 7 mL) at rt was added **9E** (260 mg, 0.94 mmol),  $\text{Cs}_2\text{CO}_3$  (614 mg, 1.89 mmol) and a solution of  $\text{Pd}_2(\text{dba})_3$  and (S)-N,N-dimethyl-1-[(R)-2-(diphenylphosphino)ferrocenyl] ethylamine (1:6 ratio, 0.06 mol%) in nitrogen degassed toluene (3 mL). The reaction was degassed with nitrogen for 30 minutes, sealed and heated at 110 °C for 48 h. Cool to rt and extract with ethylacetate (50 mL)  
 15 and wash with water followed by sat. aqueous  $\text{NaHCO}_3$ . Dry over  $\text{MgSO}_4$ , filter and concentrate. Purification by flash chromatography (40 g ISCO silica gel cartridge, 0-10% ethylacetate in hexane gradient) gave the title compound (460 mg): MS (ES)  $m/z$  494.29  $[M+H]^+$

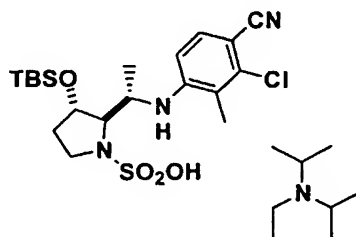
20 **9G. (2R,3S)-3-(tert-Butyldimethylsilanyloxy)-2-[(1S)-1-(3-chloro-4-cyano-2-methyl-phenylamino)-ethyl]pyrrolidine, trifluoroacetic acid salt**



Intermediate **9F** (825 mg, 1.67 mmol) was dried azeotropically with toluene  
 25 (2x). The residue was taken up in 15% TFA in  $\text{CH}_2\text{Cl}_2$  (10 mL) and stirred at rt for 5

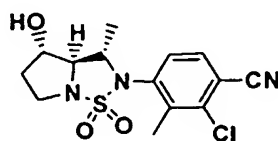
h. Toluene (10 mL) was added and solvent was removed under vacuum. The product was purified by reverse phase HPLC (Phenoenex Luna 30 x 100 mm S5 C18, 10 min. grad, 25 mL/min, 20-100%B solvent, A = 10% MeOH/water + 0.1%TFA, B = 90% MeOH/Water + 0.1%TFA, 4 injections) . Solvent was reduced to ~10% volume and the product was then extracted into EtOAc, washed with sat. aqueous NaHCO<sub>3</sub>, dried over MgSO<sub>4</sub> and filtered. Solvents were removed to provide the title compound (371 mg): MS (ES) *m/z* 394.53 [M+H]<sup>+</sup>

9H. (2R,3S)-3-(tert-Butyldimethylsilanyloxy)-2-[(1S)-1-(3-chloro-4-cyano-2-methyl-phenylamino)-ethyl]pyrrolidine-1-sulfonic acid, *N,N,N*-diisopropyl ethylamine salt



To a solution of intermediate 9G (75 mg, 0.071 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) at rt was added iPr<sub>2</sub>Net (150 μL). To this mixture was added a solution of 10% chlorosulfonic acid in CH<sub>2</sub>Cl<sub>2</sub> (200 μL). After 5 minutes the progress of the reaction was checked by LCMS – monitoring the disappearance of starting material and the production of the intermediate sulfamic acid. An additional aliquot of 10% chlorosulfonic acid in CH<sub>2</sub>Cl<sub>2</sub> (50 μL) was added every 10 minutes, checking progress by LCMS shortly after addition, until starting material had be consumed. Toluene was added to the mixture and solvent was removed to provide the title compound as a light brown residue: MS (ES) *m/z* 474.40 [M+H]<sup>+</sup>

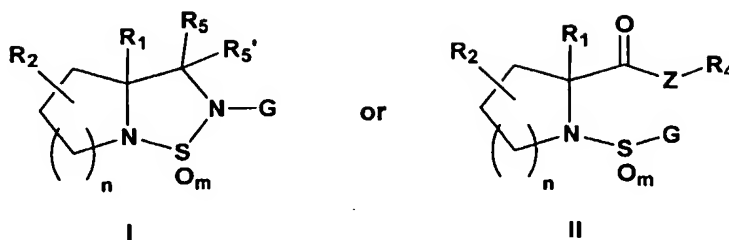
9I. 4-[(3*aR*, 3*S*,4*S*)-4-Hydroxy-3-methyl-1,1-dioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile



To a solution of intermediate **9H** (crude residue from above) in CH<sub>2</sub>Cl<sub>2</sub> (2 mL) and pyridine (200 µL) was added a solution of 10% POCl<sub>3</sub> in CH<sub>2</sub>Cl<sub>2</sub> (200 µL). After 20 minutes the progress of the reaction was checked by LCMS – monitoring the disappearance of starting material and the production of the intermediate sulfamoyl chloride and sulfamide. An additional aliquot of 10% POCl<sub>3</sub> in CH<sub>2</sub>Cl<sub>2</sub> (50 µL) was added every 10 minutes, checking progress by LCMS shortly after addition, until starting material had been consumed. Reaction was then heated at 50 °C overnight. Reaction was diluted into ethylacetate and washed with sat. aqueous NaHCO<sub>3</sub> and water. The organic phase was dried over MgSO<sub>4</sub>, filtered and concentrated to give a brown residue. The residue was taken up into THF (1 mL) and treated with TBAF (0.5 mL) for 4 hr. Solvents were removed and purification by Prep. HPLC (Phenomenex 20x250 mm S5 C18, 20 mL/min, 20 min. gradient, 20-100%B, A = 10% MeOH/water + 0.1%TFA, B = 90% MeOH/Water + 0.1%TFA) provided the title compound (28 mg): MS (ES) *m/z* 342.28 [M+H]<sup>+</sup>

## WHAT IS CLAIMED IS:

1. A compound having the formula



5

wherein

$R_1$  is hydrogen (H), alkyl or substituted alkyl, alkenyl or substituted alkenyl, arylalkyl or substituted arylalkyl,  $\text{CO}_2\text{R}_{4a}$ ,  $\text{CONR}_{4a}\text{R}_{4b}$ , or  $\text{CH}_2\text{OR}_{4a}$ ;

10  $R_2$  is hydrogen (H),  $\text{OR}_3$ ,  $\text{SR}_3$ , halo,  $\text{NHR}_3$ ,  $\text{NHCOR}_{4c1}$ ,  $\text{NHCO}_2\text{R}_{4c1}$ ,  $\text{NHCONR}_{4c}\text{R}_{4d}$  or  $\text{NHSO}_2\text{R}_{4c}$ ;

$R_3$  in each functional group is independently hydrogen (H), alkyl or substituted alkyl,  $\text{CHF}_2$ ,  $\text{CF}_3$  or  $\text{COR}_{4e}$ ;

15  $R_4$ ,  $R_{4a}$ ,  $R_{4b}$ ,  $R_{4c}$ ,  $R_{4c1}$ ,  $R_{4d}$ ,  $R_{4e}$ ,  $R_{4f}$ ,  $R_{4g}$ , and  $R_{4h}$  in each functional group are the same or different and are each independently hydrogen(H), alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, arylalkyl or substituted arylalkyl, aryl or substituted aryl, or heteroaryl or substituted heteroaryl;

20  $R_5$  and  $R_{5'}$  are the same or different and are each independently selected from the group consisting of hydrogen(H), alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, arylalkyl or substituted arylalkyl, aryl or substituted aryl, or heteroaryl or substituted heteroaryl, wherein at least one of  $R_5$  and  $R_{5'}$  is hydrogen, or  $R_5$  and  $R_{5'}$  taken together form a double bond with oxygen (O), sulfur (S),  $\text{NR}_7$  or  $\text{CR}_7\text{R}_{7'}$ ;

25  $R_7$  and  $R_{7'}$  in each functional group are the same or different and are each independently hydrogen(H),  $\text{OR}_4$ , alkyl or substituted alkyl, alkenyl or substituted

alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, arylalkyl or substituted arylalkyl, aryl or substituted aryl, or heteroaryl or substituted heteroaryl;

G is an aryl, heterocyclo or heteroaryl group, wherein said group is mono- or polycyclic, and which is optionally substituted with one or more substituents selected  
 5 from the group consisting of hydrogen, halo, CN, CF<sub>3</sub>, OR<sub>4f</sub>, CO<sub>2</sub>R<sub>4f</sub>, NR<sub>4f</sub>R<sub>4g</sub>, CONR<sub>4f</sub>R<sub>4g</sub>, CH<sub>2</sub>OR<sub>4f</sub>, alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, arylalkyl or substituted arylalkyl, aryl or substituted aryl, or heteroaryl or substituted heteroaryl; and

10 n is an integer of 1 or 2;

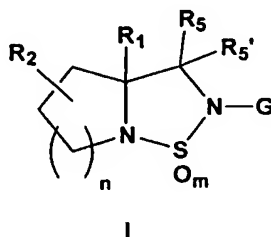
m is an integer of 1 or 2;

Z is oxygen (-O-) or NR<sub>4h</sub>;

a prodrug ester, all stereoisomers thereof and a pharmaceutically acceptable salt.

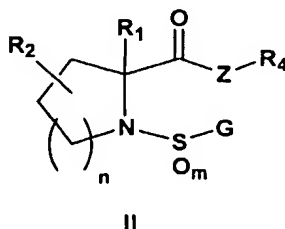
15

2. The compound as defined in Claim 1 having the formula



20

3. The compound as defined in Claim 1 having the formula II





4. The compound as defined in Claim 2 wherein

$R_1$  is H or alkyl,

$R_2$  is OH;

$R_5$  and  $R_5'$  are each H or  $R_5$  and  $R_5'$  are taken together form a double bond

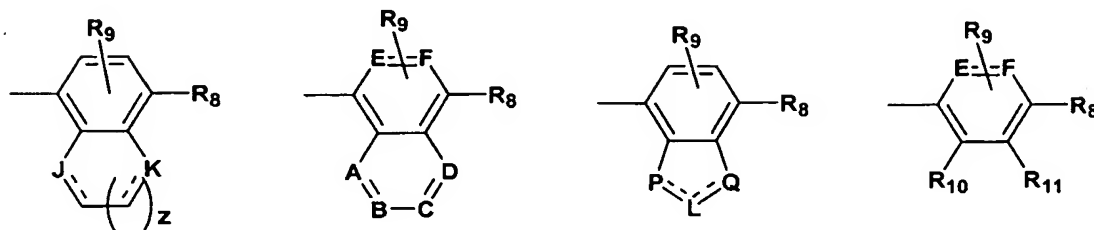
5 with oxygen or sulfur;

$n$  is 1;

$m$  is 2.

5. The compound as defined in Claim 2 wherein G is selected from:

10



wherein

15  $R_8$ ,  $R_9$ ,  $R_{10}$  and  $R_{11}$  in each functional group are the same or different and are each independently selected from the group consisting of hydrogen (H),  $\text{NO}_2$ , CN,  $\text{CF}_3$ ,  $\text{OR}_{4i}$ ,  $\text{CO}_2\text{R}_{4i}$ ,  $\text{NR}_{4i}\text{R}_{4j}$ ,  $\text{CONR}_{4i}\text{R}_{4j}$ ,  $\text{CH}_2\text{OR}_{4i}$ , alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, arylalkyl or substituted arylalkyl, aryl or substituted aryl, or heteroaryl or substituted heteroaryl;

20 A to F is each independently selected from N or  $\text{CR}_{1a}$ ;

J, K, L, P and Q are each independently selected from  $\text{NR}_{12}$ , O, S, SO,  $\text{SO}_2$  or  $\text{CR}_{12}\text{R}_{12'}$ ;

$R_{12}$  and  $R_{12'}$  in each functional group are each independently selected from a bond or  $\text{R}_{1b}$ ;

25  $R_{1a}$  and  $R_{1b}$  are each independently selected from H, alkyl or substituted alkyl, alkenyl or substituted alkenyl, arylalkyl or substituted arylalkenyl,  $\text{CO}_2\text{R}_{4k}$ ,  $\text{CONR}_{4k}\text{R}_{4l}$  and  $\text{CH}_2\text{OR}_{4k}$ ; and

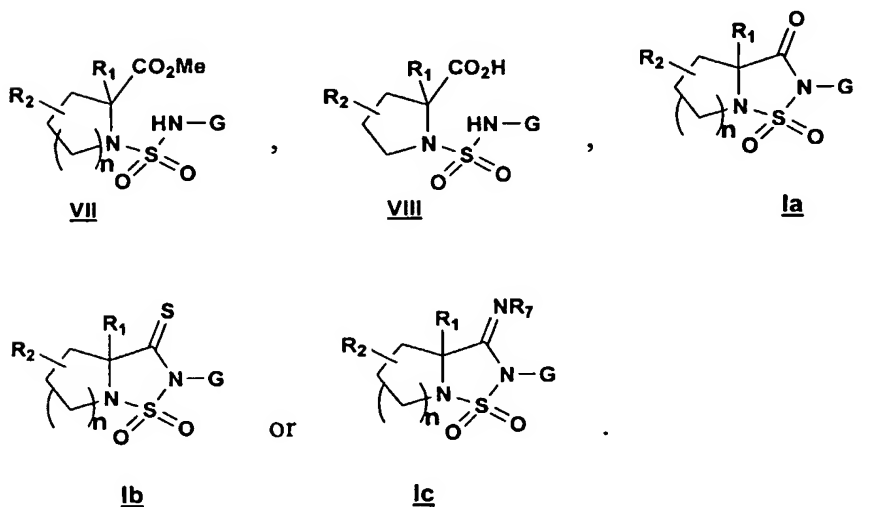
$R_{4i}$ ,  $R_{4j}$ ,  $R_{4k}$  and  $R_{4l}$  in each functional group are the same or different and are each independently hydrogen(H), alkyl or substituted alkyl, alkenyl or substituted alkenyl, alkynyl or substituted alkynyl, cycloalkyl or substituted cycloalkyl, arylalkyl or substituted arylalkyl, aryl or substituted aryl, or heteroaryl or substituted heteroaryl;

5  $z$  is an integer of 0 or 1.

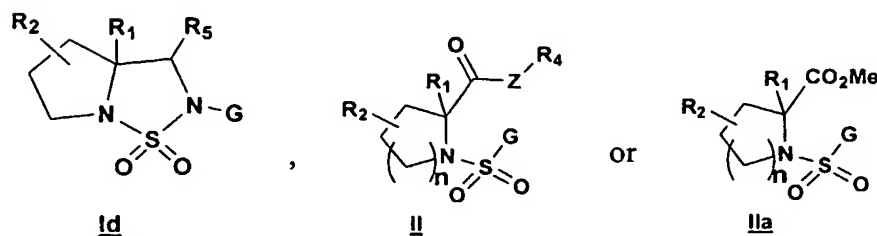
6. The compound as defined in Claim 5 wherein  $R_8$  is CN.

7. The compound as defined in Claim 1 having the structure

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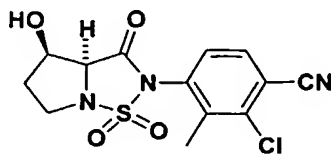


15 8. The compound as defined in Claim 1 having the structure



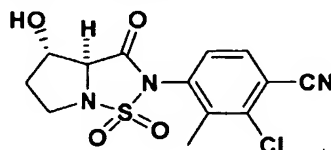
9. The compound as defined in Claim 1 having the name and structure

**4-[(3a*S*,4*R*)-4-Hydroxy-1,1,3-trioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile**



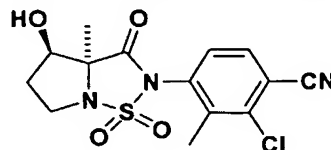
5

**4-[(3a*S*,4*S*)-4-Hydroxy-1,1,3-trioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile**

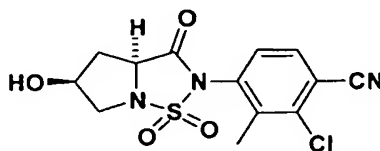


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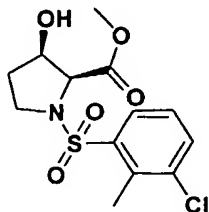
**4-[(3a*S*,4*R*)-4-Hydroxy-3a-methyl-1,1,3-trioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile**



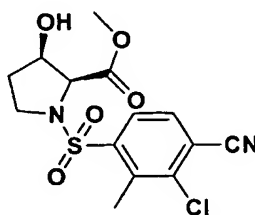
15 **4-[(3a*S*,5*S*)-5-Hydroxy-1,1,3-trioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile**



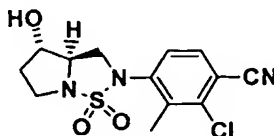
**(2S,3R)-1-(3-Chloro-2-methyl-benzenesulfonyl)-3-hydroxy-pyrrolidine-2-carboxylic acid methyl ester**



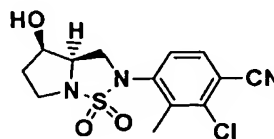
**5 (2S,3R)-1-(3-Chloro-4-cyano-2-methyl-benzenesulfonyl)-3-hydroxy-pyrrolidine-2-carboxylic acid methyl ester**



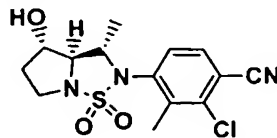
**10 4-[(3aR,4S)-4-Hydroxy-1,1-dioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile**



**2-Chloro-4-[(3aR,4R)-4-hydroxy-1,1-dioxohexahydrothia-2,6a-diazapentalen-2-yl]-3-methylbenzonitrile**



**15 4-[(3aR, 3S,4S)-4-Hydroxy-3-methyl-1,1-dioxohexahydrothia-2,6a-diazapentalen-2-yl]-2-chloro-3-methylbenzonitrile**



10. A method for treating androgen receptor-associated conditions, which comprises administering to a patient in need of treatment a therapeutically effective amount of a compound as defined in Claim 1.
- 5
11. The method as defined in Claim 10 wherein the compounds employed are selective agonists, partial agonists, antagonists or partial antagonists of the androgen receptor.
- 10
12. The method as defined in Claim 10 wherein the compounds employed are agonists of the androgen receptor.
13. The method as defined in Claim 12 wherein the compounds are used in maintenance of muscle strength and function; reversal or prevention of frailty or age-related functional decline ("ARFD") in the elderly; prevention of catabolic side effects of glucocorticoids; prevention and treatment of reduced bone density or growth in the treatment of osteoporosis, and osteopenia; treatment of chronic fatigue syndrome (CFS); chronic myalgia; treatment of acute fatigue syndrome and muscle loss following elective surgery; acceleration of wound healing; accelerating bone fracture repair; treatment of wasting secondary to fractures and wasting in connection with chronic obstructive pulmonary disease (COPD), chronic liver disease, AIDS, weightlessness, cancer cachexia, burn and trauma recovery, chronic catabolic state, coma, eating disorders, anorexia, and chemotherapy.
- 15
- 20
- 25
14. The method as defined in Claim 10 wherein the compounds employed are in a composition employing such compounds and a pharmaceutically acceptable carrier therefor.
- 15.
- 30
15. The method as defined in Claim 10 comprising administering a therapeutically effective amount of the compound alone or in combination with another therapeutic agent.

16. A method for preventing, inhibiting or treating the progression or onset of diseases or disorders associated with nuclear hormone receptors, wherein a therapeutically effective amount of a compound as defined in Claim 1 is administered to a mammalian patient in need of treatment.

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17. A method for the maintenance of muscle strength and function; reversal or prevention of frailty or age-related functional decline; treatment of catabolic side effects of glucocorticoids; prevention and/or treatment of reduced bone mass, density or growth; osteoporosis and osteopenia; treatment of chronic fatigue syndrome (CFS); chronic myalgia; treatment of acute fatigue syndrome and muscle loss following elective surgery; accelerating of wound healing; accelerating bone fracture repair; accelerating healing of complicated fractures, distraction osteogenesis; in joint replacement; prevention of post-surgical adhesion formation; acceleration of tooth repair or growth; maintenance of sensory function; treatment of periodontal disease; treatment of wasting secondary to fractures and wasting in connection with chronic obstructive pulmonary disease (COPD), chronic liver disease, AIDS, weightlessness, cancer cachexia, burn and trauma recovery, chronic catabolic state; eating disorders; treatment of cardiomyopathy; treatment of thrombocytopenia; treatment of growth retardation in connection with Crohn's disease; treatment of short bowel syndrome; treatment of irritable bowel syndrome; treatment of inflammatory bowel disease; treatment of Crohn's disease and ulcerative colitis; treatment of complications associated with transplantation; treatment of physiological short stature including growth hormone deficient children and short stature associated with chronic illness; treatment of obesity and growth retardation associated with obesity; treatment of anorexia; treatment of hypercortisolism and Cushing's syndrome; Paget's disease; treatment of osteoarthritis; induction of pulsatile growth hormone release; treatment of osteochondrodysplasias; treatment of depression, nervousness, irritability and stress; treatment of reduced mental energy and low self-esteem; improvement of cognitive function, the treatment of dementia, Alzheimer's disease and short term memory loss; treatment of catabolism in connection with pulmonary dysfunction and ventilator dependency; treatment of cardiac dysfunction associated with valvular disease, myocardial infarction, cardiac hypertrophy or congestive heart failure;

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lowering blood pressure; protection against ventricular dysfunction or prevention of reperfusion events; treatment of adults in chronic dialysis; reversal or slowing of the catabolic state of aging; attenuation or reversal of protein catabolic responses following trauma, reversal of the catabolic state associated with surgery, congestive heart failure, cardiac myopathy, burns, cancer, COPD; reducing cachexia and protein loss due to chronic illness such as cancer or AIDS; treatment of hyperinsulinemia including nesidioblastosis; treatment of immunosuppressed patients; treatment of wasting in connection with multiple sclerosis or other neurodegenerative disorders; promotion of myelin repair; maintenance of skin thickness; treatment of metabolic homeostasis and renal homeostasis; stimulation of osteoblasts, bone remodeling and cartilage growth; regulation of food intake; treatment of insulin resistance, including NIDDM, in mammals; treatment of insulin resistance in the heart; improvement of sleep quality and correction of the relative hyposomatotropism of senescence due to high increase in REM sleep and a decrease in REM latency; treatment of hypothermia; treatment of congestive heart failure; treatment of lipodystrophy; treatment of muscular atrophy; treatment of musculoskeletal impairment; improvement of the overall pulmonary function; treatment of sleep disorders; and the treatment of the catabolic state of prolonged critical illness; treatment of hirsutism, acne, seborrhea, androgenic alopecia, anemia, hyperpilosity, benign prostate hypertrophy, adenomas and neoplasies of the prostate and malignant tumor cells containing the androgen receptor, as is the case for breast, brain, skin, ovarian, bladder, lymphatic, liver and kidney cancers; cancers of the skin, pancreas, endometrium, lung and colon; osteosarcoma; hypercalcemia of malignancy; metastatic bone disease; treatment of spermatogenesis, endometriosis and polycystic ovary syndrome; counteracting preeclampsia, eclampsia of pregnancy and preterm labor; treatment of premenstrual syndrome; treatment of vaginal dryness; age related decreased testosterone levels in men, male menopause, hypogonadism, male hormone replacement, male and female sexual dysfunction, erectile dysfunction, decreased sex drive, sexual well-being, decreased libido, urinary incontinence, male and female contraception, hair loss, Reaven's Syndrome and the enhancement of bone and muscle performance/strength, and "Syndrome X" or Metabolic Syndrome, which comprises treating a human patient

in need of treatment with a therapeutic amount of a compound as defined in Claim 1 alone or in combination with another therapeutic agent.

18. The method as defined in Claim 17 wherein the other therapeutic agent
- 5 is an antibiotic, or growth promoting agent, TRH, diethylstilbesterol, theophylline, enkephalins, E series prostaglandins, zeranol, sulbenox, growth hormone secretagogues, GHRP-6, GHRP-1, GHRP-2, NN703 (Novo Nordisk), LY444711 (Lilly), MK-677 (Merck), CP424391 (Pfizer) and B-HT920, or with growth hormone releasing factor and its analogs or growth hormone and its analogs or somatomedins,
- 10 IGF-1 and IGF-2, or with alpha-adrenergic agonists, clonidine or serotonin 5-HT<sub>D</sub> agonists, sumatriptan, or agents which inhibit somatostatin or its release, physostigmine and pyridostigmine, parathyroid hormone, PTH(1-34) or bisphosphonates, MK-217 (alendronate), testosterone, a selective estrogen receptor modulator, tamoxifen or raloxifene, other androgen receptor modulators, progesterone
- 15 receptor agonist ("PRA"), which is levonorgestrel, medroxyprogesterone acetate (MPA), other modulators of nuclear hormone receptors, an anti-diabetic agent; anti-osteoporosis agent; anti-obesity agent; anti-inflammatory agent; anti-anxiety agent; anti-depressant; anti-hypertensive agent; anti-platelet agent; anti-thrombotic and thrombolytic agents; cardiac glycoside; cholesterol/lipid lowering agent;
- 20 mineralocorticoid receptor antagonist; phosphodiesterase inhibitor; protein tyrosine kinase inhibitor; thyroid mimetic and a thyroid receptor agonist; anabolic agent; HIV or AIDS therapy; a therapy useful in the treatment of Alzheimer's disease and other cognitive disorders; a therapy useful in the treatment of sleeping disorders; anti-proliferative agent; and anti-tumor agent; wherein the anti-diabetic agent is a
- 25 biguanide, metformin, glucosidase inhibitor, acarbose, insulin, insulin secretagogue and insulin sensitizer, meglitinide, repaglinide, sulfonylurea, glimepiride, glyburide and glipizide, biguanide/glyburide combination, thiazolidinedione, troglitazone, rosiglitazone and pioglitazone, PPAR-alpha agonist, PPAR-gamma agonist, PPAR alpha/gamma dual agonist, SGLT2 inhibitor, glycogen phosphorylase inhibitor,
- 30 inhibitors of fatty acid binding protein (aP2), glucagon-like peptide-1 (GLP-1), and dipeptidyl peptidase IV (DPP4) inhibitor, anti-osteoporosis agent which is alendronate, risedronate, PTH, PTH fragment, raloxifene, calcitonins, steroidal or



non-steroidal progesterone receptor agonists, RANK ligand antagonist, calcium sensing receptor antagonist, TRAP inhibitor, selective estrogen receptor modulator (SERM's), estrogen and AP-1 inhibitor, anti-obesity agent which is aP2 inhibitor, PPAR gamma antagonist, PPAR delta agonist, beta 3 adrenergic agonist, AJ9677  
5 (Takeda/Dainippon), L750355 (Merck), or CP331648 (Pfizer), a lipase inhibitor, orlistat or ATL-962 (Alizyme), a serotonin (and dopamine) reuptake inhibitor, sibutramine, topiramate or axokine, a thyroid receptor beta drug, a thyroid receptor ligand and GB98/284425 (KaroBio), and/or an anorectic agent, dexamphetamine, phentermine, phenylpropanolamine or mazindol, anti-inflammatory agent which is  
10 prednisone, dexamethasone, Enbrel<sup>®</sup>, cyclooxygenase inhibitors, COX-1 and/or COX-2 inhibitors, NSAIDs, aspirin, indomethacin, ibuprofen, piroxicam, Naproxen<sup>®</sup>, Celebrex<sup>®</sup>, Vioxx<sup>®</sup>, CTLA4-Ig agonists/antagonist, CD40 ligand antagonist, IMPDH inhibitor, mycophenolate (CellCept<sup>®</sup>), integrin antagonist, alpha-4 beta-7 integrin antagonist, cell adhesion inhibitor, interferon gamma antagonist, ICAM-1, tumor  
15 necrosis factor (TNF) antagonist, infliximab, OR1384, prostaglandin synthesis inhibitor, budesonide, clofazimine, CNI-1493, CD4 antagonist, priliximab, p38 mitogen-activated protein kinase inhibitor, protein tyrosine kinase (PTK) inhibitor, IKK inhibitor, and therapy for the treatment of irritable bowel syndrome, Zelmac<sup>®</sup> and Maxi-K<sup>®</sup> opener; anti-anxiety agent which is diazepam, lorazepam, buspirone,  
20 oxazepam, and hydroxyzine pamoate; anti-depressant which is citalopram, fluoxetine, nefazodone, sertraline, and paroxetine; anti-hypertensive agent which is beta adrenergic blocker, calcium channel blocker, L-type and T-type, diltiazem, verapamil, nifedipine, amlodipine and mybefradil, diuretic, chlorothiazide, hydrochlorothiazide, flumethiazide, hydroflumethiazide, bendroflumethiazide, methylchlorothiazide,  
25 trichloromethiazide, polythiazide, benzthiazide, ethacrynic acid tricrynafen, chlorthalidone, furosemide, musolimine, bumetanide, triamtrenene, amiloride, spironolactone, renin inhibitor, ACE inhibitor, captopril, zofenopril, fosinopril, enalapril, ceranopril, cilazopril, delapril, pentopril, quinapril, ramipril, lisinopril, AT-1 receptor antagonist, losartan, irbesartan, valsartan, ET receptor antagonist,  
30 sitaxsentan, atrsentan, Dual ET/AII antagonist, neutral endopeptidase (NEP) inhibitor, vasopepsidase inhibitor, dual NEP-ACE inhibitor, omapatrilat and gemopatrilat, and

nitrate, anti-platelet agent which is a GPIIb/IIIa blocker, abciximab, eptifibatide, tirofiban, P2Y<sub>12</sub> antagonist, clopidogrel, ticlopidine, CS-747, thromboxane receptor antagonist, ifetroban, aspirin, and PDE-III inhibitors, dipyridamole, cardiac glycoside which is digitalis and ouabain; cholesterol/lipid lowering agent which is HMG-CoA reductase inhibitor, pravastatin, lovastatin, atorvastatin, simvastatin, NK-104 or itavastatin, or nisvastatin or nisbastatin, ZD-4522 or rosuvastatin, or atavastatin or visastatin, squalene synthetase inhibitor, fibrate, bile acid sequestrant, ACAT inhibitor, MTP inhibitor, lipooxygenase inhibitor, cholesterol absorption inhibitor, and cholesterol ester transfer protein inhibitor, CP-529414, mineralocorticoid receptor antagonist which is spironolactone and eplerenone, phosphodiesterase (PDE) inhibitor which is PDE-3 inhibitors, cilostazol, and phosphodiesterase-5 inhibitor (PDE-5 inhibitors, such as sildenafil; thyroid mimetics which is thyrotropin, polythyroid, KB-130015, and dronedarone; anabolic agent which is testosterone, TRH diethylstilbesterol, estrogen,  $\beta$ -agonist, theophylline, anabolic steroid, dehydroepiandrosterone, an enkephalin, E-series prostaglandin, retinoic acid, Zeranol<sup>®</sup>, Sulbenox<sup>®</sup>; HIV or AIDS therapy which is indinavir sulfate, saquinavir, saquinavir mesylate, ritonavir, lamivudine, zidovudine, lamivudine/zidovudine combinations, zalcitabine, didanosine, stavudine, and megestrol acetate; treatment of Alzheimer's disease and cognitive disorders which is donepezil, tacrine, revastigmine, 5HT<sub>6</sub>, gamma secretase inhibitors, beta secretase inhibitors, SK channel blockers, Maxi-K blockers, and KCNQs blockers; therapy for treatment of sleeping disorders which is a melatonin analog, melatonin receptor antagonist, ML1B agonist, and GABA/NMDA receptor antagonist; anti-proliferative agents which is cyclosporin A, paclitaxel, FK-506, and adriamycin; anti-tumor agent which is paclitaxel, adriamycin, epothilones, cisplatin and carboplatin; a nutritional supplement which is whey protein or casein, an amino acid such as leucine, branched amino acid and hydroxymethylbutyrate, a triglyceride, vitamin A, B<sub>6</sub>, B<sub>12</sub>, folate, C, D and E, mineral which is selenium, magnesium, zinc, chromium, calcium and potassium, carnitine, lipoic acid, creatinine, B-hydroxy-B-methylbutyrate (Juven) and coenzyme Q-10; a therapeutic agent used in the treatment of sexual dysfunction which is a PDE-5 inhibitor, sildenafil or IC-351; an antiresorptive agent, hormone replacement therapy, vitamin D analogue, elemental

calcium and calcium supplement, cathepsin K inhibitor, MMP inhibitor, vitronectin receptor antagonist, Src SH<sub>2</sub> antagonist, vacuolar H<sup>+</sup>-ATPase inhibitor, ipriflavone, fluoride, Tibolone, prostanoid, 17-beta hydroxysteroid dehydrogenase inhibitor and Src kinase inhibitor; a male contraceptive which is nonoxynol 9, a therapeutic agent  
5 for the treatment of hair loss which is minoxidil and finasteride or a chemotherapeutic agent which is an LHRH agonist; anti-cancer and cytotoxic agent which is alkylating agent, nitrogen mustard, alkyl sulfonate, nitrosourea, ethylenimine, and triazene; antimetabolite which is a folate antagonist, purine analogue, and pyrimidine analogue; antibiotic which is anthracycline, bleomycin, mitomycin, dactinomycin, and  
10 plicamycin; an enzyme which is L-asparaginase; farnesyl-protein transferase inhibitor; 5 $\alpha$ -reductase inhibitor; inhibitor of 17 $\beta$ -hydroxysteroid dehydrogenase type 3; hormonal agent which is a glucocorticoid, estrogen/antiestrogen, androgen/antiandrogen, progestin, and luteinizing hormone-releasing hormone antagonist, octreotide acetate; microtubule-disruptor agent which is an ecteinascidin  
15 or their analogs and derivative; microtubule-stabilizing agent which is a taxane, paclitaxel (Taxol<sup>®</sup>), docetaxel (Taxotere<sup>®</sup>), and their analogs, and an epothilone which is epothilones A-F and their analogs; a plant-derived product which is vinca alkaloids, epipodophyllotoxin, a taxane; and a topoisomerase inhibitor; prenyl-protein transferase inhibitor; hydroxyurea, procarbazine, mitotane, hexamethylmelamine, a  
20 platinum coordination complex, cisplatin and carboplatin; a biological response modifier, growth factor; immune modulator and monoclonal antibody and radiation therapy; an anti-cancer and cytotoxic agent which is mechlorethamine hydrochloride, cyclophosphamide, chlorambucil, melphalan, ifosfamide, busulfan, carmustin, lomustine, semustine, streptozocin, thiotepa, dacarbazine, methotrexate, thioguanine,  
25 mercaptopurine, fludarabine, pentastatin, cladribin, cytarabine, fluorouracil, doxorubicin hydrochloride, daunorubicin, idarubicin, bleomycin sulfate, mitomycin C, actinomycin D, safracins, saframycins, quinocarcins, discodermolides, vincristine, vinblastine, vinorelbine tartrate, etoposide, etoposide phosphate, teniposide, paclitaxel, tamoxifen, estramustine, estramustine phosphate sodium, flutamide,  
30 buserelin, leuprolide, pteridines, diynes, levamisole, aflacon, interferon, interleukins, aldesleukin, filgrastim, sargramostim, rituximab, BCG, tretinoin, irinotecan hydrochloride, betamethosone, gemcitabine hydrochloride, altretamine, and

topoteca and any analogs or derivatives thereof; an epothilone derivative; cyclin dependent kinase inhibitors; and prenyl-protein transferase inhibitor, NHR modulator, AR modulator, ER modulator, LHRH modulator, or with surgical castration.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US05/02834

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07D 285/06; A61K 31/433

US CL : 548/127; 514/361

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 548/127; 514/361

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
STN Databases Online: FILE REG, FILE CAPLUS (structure search, etc); EAST; WEST

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	U.S. 5,556,909 (DESAI) 17 September 1996 (17.09.1996), see entire document.	9

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

02 May 2005 (02.05.2005)

Date of mailing of the international search report

13 MAY 2005

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US05/02834

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 1-8 and 10-18  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
Please See Continuation Sheet
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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The additional search fees were accompanied by the applicant's protest.

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No protest accompanied the payment of additional search fees.

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## Continuation of Box II Reason 2:

In these claims, the numerous variables (e.g. R1-R7, G, Z, m, n, etc), their voluminous complex meanings, their seemingly endless permutations and combinations make it virtually impossible to determine the full scope and complete meaning of the claimed subject matter. As presented, the claimed subject matter cannot be regarded as being a clear and concise description for which protection is sought and as such the listed claims do not comply with the requirements of PCT article 6. Thus it is impossible to carry out a meaningful search on the same. A search will be made on the first discernable invention in the claims, which is the first recited compound of claim 9.